



sau96I  
 avall  
 asul  
 fnu4HI      accI      nlaIV  
 286 GTA GCG GCG GAA GTC TAC GGG ACC GAA AGA CAG CCA CGA ACT CAC TAT  
 78 Val Ala Ala Glu Val Tyr Gly Thr Glu Arg Gln Pro Arg Thr His Tyr

fnu4HI  
 mboII      bbvI      aluI  
 hphI      fnu4HI      aluI      pvuII  
 334 TAT GCC GTG GCT GTG GTG AAG AAG GGC GGC AGC TTT CAG CTG AAC GAA  
 94 Tyr Ala Val Ala Val Val Lys Lys Gly Gly Ser Phe Gln Leu Asn Glu

haeIII      sau96I  
 stuI      avall  
 bglI      haeI      asul      foki  
 382 CTG CAA GGT CTG AAG TCC TGC CAC ACA GGC CTT CGC AGG ACC GCT GGA  
 110 Leu Gln Gly Leu Lys Ser Cys His Thr Gly Leu Arg Arg Thr Ala Gly

sau96I  
 avall  
 asul  
 nlaIV  
 430 TGG AAT GTC CCT ACA GGG ACA CTT CGT CCA TTC TTG AAT TGG ACG GGT  
 126 Trp Asn Val Pro Thr Gly Thr Leu Arg Pro Phe Leu Asn Trp Thr Gly

hgiIII      aluI  
 bsp1286      fnu4HI  
 banII      bbvI  
 ddeI      mboII      pvuII      mboII      aluI  
 478 CCA CCT GAG CCC ATT GAG GCA GCT GTG CAG TTC TTC TCA GCC AGC TGT  
 142 Pro Pro Glu Pro Ile Glu Ala Ala Val Gln Phe Phe Ser Ala Ser Cys

mspI  
 hpaII  
 scrFI  
 nciI  
 cauII  
 526 GTT CCC GGT GCA GAT AAA GGA CAG TTC CCC AAC CTG TGT CGC CTG TGT  
 158 Val Pro Gln Ala Asp Lys Gly Gln Phe Pro Asn Leu Cys Arg Leu Cys

nlaIV  
 scrFI  
 ecoRII  
 mnlI      bstNI      rsaI  
 574 GCG GGG ACA GGG GAA AAC AAA TGT GCC TTC TCC TCC CAG GAA CCG TAC  
 174 Ala Gly Thr Gly Glu Asn Lys Cys Ala Phe Ser Ser Gln Glu Pro Tyr

FIG.-1

nlaIV  
 hgiCI  
 aluI      banI      ddeI    bsmal      bsmal  
 622 TTC AGC TAC TCT GGT GCC TTC AAG TGT CTG AGA GAC GGG GCT GGA GAC  
 190 Phe Ser Tyr Ser Gly Ala Phe Lys Cys Leu Arg Asp Gly Ala Gly Asp  
  
 sau96I  
 avall  
 asuI  
 ppvMI  
 ecoRI109I  
 hgiAI  
 bsp1286      mnlI      mnlI  
 670 GTG GCT TTT ATC AGA GAG AGC ACA GTG TTT GAG GAC CTG TCA GAC GAG  
 206 Val Ala Phe Ile Arg Glu Ser Thr Val Phe Glu Asp Leu Ser Asp Glu  
  
 718 GCT GAA AGG GAC GAG TAT GAG TTA CTC TGC CCA GAC AAC ACT CGG AAG  
 222 Ala Glu Arg Asp Glu Tyr Glu Leu Leu Cys Pro Asp Asn Thr Arg Lys  
  
 scrFI  
 nciI  
 mspI  
 hpaII  
 caulI  
 xmaI sau96I  
 smaI nlaIV  
 scrFI  
 nciI avall  
 caulI  
 avall asuI  
 sau96I ppvMI  
 haeIII nlaIV  
 asuI    ecoRI109I      nlaIII  
 bsrI  
 766 CCA GTG GAC AAG TTC AAA GAC TGC CAT CTG GCC CGG GTC CCT TCT CAT  
 238 Pro Val Asp Lys Phe Lys Asp Cys His Leu Ala Arg Val Pro Ser His  
  
 sfaNI  
 foki      mboII  
 bglI      draIII      mnlI      hinfI  
 814 GCC GTT GTG GCA CGA AGT GTG AAT GGC AAG GAG GAT GCC ATC TGG AAT  
 254 Ala Val Val Ala Arg Ser Val Asn Gly Lys Glu Asp Ala Ile Trp Asn  
  
 scrFI  
 ecoRII  
 bstNI      hphI  
 862 CTT CTC CGC CAG GCA CAG GAA AAG TTT GGA AAG GAC AAG TCA CCG AAA  
 270 Leu Leu Arg Gln Ala Gln Glu Lys Phe Gly Lys Asp Lys Ser Pro Lys

FIG.-1

910 TTC CAG CTC TTT GGC TCC CCT AGT GGG CAG AAA GAT CTG CTG TTC AAG  
 286 Phe Gln Leu Phe Gly Ser Pro Ser Gly Gln Lys Asp Leu Leu Phe Lys

958 GAC TCT GCC ATT GGG TTT TCG AGG GTG CCC CCG AGG ATA GAT TCT GGG  
 302 Asp Ser Ala Ile Gly Phe Ser Arg Val Pro Pro Arg Ile Asp Ser Gly

1006 CTG TAC CTT GGC TCC GGC TAC TTC ACT GCC ATC CAG AAC TTG AGG AAA  
 318 Leu Tyr Leu Gly Ser Gly Tyr Phe Thr Ala Ile Gln Asn Leu Arg Lys

1054 AGT GAG GAG GAA GTG GCT GCC CGG CGT GCG CGG GTC GTG TGG TGT GCG  
 344 Ser Glu Glu Glu Val Ala Ala Arg Arg Ala Arg Val Val Trp Cys Ala

1102 GTG GGC GAG CAG GAG CTG CGC AAG TGT AAC CAG TGG AGT GGC TTG AGC  
 350 Val Gly Glu Gln Glu Leu Arg Lys Cys Asn Gln Trp Ser Gly Leu Ser

1150 GAA GGC AGC GTG ACC TGC TCC TCG GCC TCC ACC ACA GAG GAC TGC ATC  
 366 Glu Gly Ser Val Thr Cys Ser Ser Ala Ser Thr Thr Glu Asp Cys Ile

1198 GCC CTG GTG CTG AAA GGA GAA GCT GAT GCC ATG AGT TTG GAT GGA GGA  
 382 Ala Leu Val Leu Lys Gly Glu Ala Asp Ala Met Ser Leu Asp Gly Gly

FIG.-1

hphI bspI286  
 1582 GAC GAG CAG GGT GAG AAT AAG TGC GTG CCC AAC AGC AAT GAG AGA TAC  
 510 Asp Glu Gln Gly Glu Asn Lys Cys Val Pro Asn Ser Asn Glu Arg Tyr

nlaIV  
 hgiCI  
 banI scrFI  
 mspI ecoRII  
 bsrI hpaII bstNI ddeI bsmI bsmal  
 1630 TAC GGC TAC ACT GGG GCT TTC CGG TGC CTG GCT GAG AAT GCT GGA GAC  
 526 Tyr Gly Tyr Thr Gly Ala Phe Arg Cys Leu Ala Glu Asn Ala Gly Asp

1678 GTT GCA TTT GTG AAA GAT GTC ACT GTC TTG CAG AAC ACT GAT GGA AAT  
 542 Val Ala Phe Val Lys Asp Val Thr Val Leu Gln Asn Thr Asp Gly Asn

fnu4HI  
 bbvI  
 hinPI  
 hhaI  
 mnlI nlaIII ddeI aluI  
 1726 AAC AAT GAG GCA TGG GCT AAG GAT TTG AAG CTG GCA GAC TTT GCG CTG  
 558 Asn Asn Glu Ala Trp Ala Lys Asp Leu Lys Leu Ala Asp Phe Ala Leu

taqI  
 mnlI  
 bglI  
 1774 CTG TGC CTC GAT GGC AAA CGG AAG CCT GTG ACT GAG GCT AGA AGC TGC  
 574 Leu Cys Leu Asp Gly Lys Arg Lys Pro Val Thr Glu Ala Arg Ser Cys

fnu4HI  
 bbvI  
 aluI  
 ddeI  
 sau96I  
 nlaIV  
 nlaIII  
 styI haeIII  
 ncoI asuI hinfI nlaIII bsmal foki  
 1822 CAT CTT GCC ATG GCC CCG AAT CAT GCC GTG GTG TCT CGG ATG GAT AAG  
 590 His Leu Ala Met Ala Pro Asn His Ala Val Val Ser Arg Met Asp Lys

fnu4HI  
 ecoNI alwNI bbvI  
 1870 GTG GAA CGC CTG AAA CAG GTG CTG CTC CAC CAA CAG GCT AAA TTT GGG  
 606 Val Glu Arg Leu Lys Gln Val Leu Leu His Gln Gln Ala Lys Phe Gly

sau3AI  
 mboI  
 dpnI  
 xhoII  
 bstYI  
 alwI  
 mspI  
 hpaII  
 scrFI  
 nciI  
 cauII  
 bsrI  
 1919 AGA AAT GGA TCT GAC TGC CCG GAC AAG TTT TGC TTA TTC CAG TCT GAA  
 622 Arg Asn Gly Ser Asp Cys Pro Asp Lys Phe Cys Leu Phe Gln Ser Glu

FIG.-1

haeIII  
 haeI  
 eaeI styl  
 cfrI pfeI ncoI  
 ddel  
 draIII ball hinfI  
 1966 ACC AAA AAC CTT CTG TTC AAT GAC AAC ACT GAG TGT CTG GCC AGA CTC  
 638 Thr Lys Asn Leu Leu Phe Asn Asp Asn Thr Glu Cys Leu Ala Arg Leu

sau96I  
 avall  
 asuI  
 nlaIII ndel sspl nlaIV  
 2014 CAT GGC AAA ACA ACA TAT GAA AAA TAT TTG GGA CCA CAG TAT GTC GCA  
 654 His Gly Lys Thr Thr Tyr Glu Lys Tyr Leu Gly Pro Gln Tyr Val Ala

scrFI  
 ecoRII  
 bstNI  
 hgiAI  
 bsp1286 mnlI mnlI  
 2062 GGC ATT ACT AAT CGT AAA AAG TGC TCA ACC TCC CCC CTC CTG GAA GCC  
 670 Gly Ile Thr Asn Leu Lys Lys Cys Ser Thr Ser Pro Leu Leu Glu Ala

ddel  
 mstII  
 mnlI  
 eco8II  
 ecoRI bsu36I  
 sau96I  
 mboII haeIII  
 mboII asuI aluI  
 2110 TGT GAA TTC CTC AGG AAG TAA AACCGAAGAA GATGGCCCAG CTCCCCAAGA  
 685 Cys Glu Phe Leu Arg Lys DC\*

styl  
 haeIII  
 sau96I  
 asuI  
 mboII scrFI  
 earI ecoRII  
 aluI bstNI  
 nlaIV  
 eco109I nlaIV  
 2161 AAGCCTCAGC CATTCACTGC CCCAGCTCT TCTCCCCAGG TGTGTTGGGG CCTTGGCTCC

ecoNI  
 fokI  
 ddel  
 2221 CCTGCTGAAG GTGGGGATTG CCCATCCATC TGCTTACAAT TCCCTGCTGT CGTCTTAGCA  
 2281 AGAAGTAAAA TGAGAAATTT TGTGTGATATT CAAAAAAA

>LENGTH: 2319

FIG.-1

1 GACTCCTAGG GGTTCGAGA CCTAGTGGG GAGAAAGAAC ATCGCAGCAG CCAGGCAGAA CCAGGACAGG TGAGGTGCAG GCTGGCTTTC CTCCTGCAGC  
 101 GCGGTGTGGA GTCCGTGCTT GCCTCAGGGC TTTTCGGAGC CTGGATCCTC AAGGAACAAG TAGACCTGGC CGCGGGGAGT GGGGAGGGAA GGGGTGCTA  
 201 TTGGGCAACA GGGGGGCANA GCCCTGAATA AAGGGGGGCA GGGCAGGGCG AAGTGCAGAG CCTTCGTTTG CCAAGTCGCC TCAGACCCGC AGAC ATG AAA CTT  
 -19 M K L  
 304 GTC TTC CTC CTC CTC CTC GGA CTG TGT CTG GCT GGC CGT AGG AGA AGG AGT GTT CAG TGG TGC GGC GTA TCC  
 -16 V F L L L F L L G A L G L C L A G R R R S V Q W C A V S  
 391 CAA CCC GAG GCC ACA AAA TGC TTC CAA TGG CAA AGG AAT ATG AGA AAA GTG CGT GGC CCT CCT GTC AGC TGC ATA AAG AGA GAC TCC  
 14 Q P E A T K C F Q V Q R N M R K V R G P P V S C I K R D S  
 478 CCC ATC CAG TGT ATC CAG GCC ATT GCG GAA AAC AGG GCC GAT GCT GTG ACC CTT GAT GGT TTT ATA TAC GAG GCA GGC CTG GCC  
 43 P I Q C I Q A I A E N R A D A V T L D G G F I Y E A G L A  
 565 CCC TAC AAA CTG CGA CCT GTA GCG GCG GAA GTC TAC GGG ACC GAA AGA CAG CCA CGA ACT CAC TAT TAT GGC GTG GCT GTG GTG AAG  
 72 P Y K L R P V A A E V Y G T E R Q P R T H Y A V A V V K  
 652 AAG GGC GGC AGC TTT CAG CTG AAC GAA CTG CAA GGT CTG AAG TCC TGC CAC ACA GGC CTT CGC AGG ACC GCT GGA TGG AAT GTC CCT  
 101 K G G S F Q L N E L Q G L K S C H T G L R R T A G W N V P  
 739 ACA GGG ACA CTT CGT CCA TTC TTG AAT TGG ACG GGT CCA CCT GAG CCC ATT GAG GCA GCT GTG GCC AGG TTC TCA GCC AGC TGT  
 130 T G T L R P F L N W T G P P E P I E A A V A R F S A S C  
 826 GTT CCC GCA GAT AAA GGA CAG TTC CCC AAC CTG TGT CGC CTG TGT GCG GGG ACA GGG GAA AAC AAA TGT GCT TTC TCC TCC CAG  
 159 V P G A D K G Q F P N L C R L C A G T G E N K C A F S S Q  
 913 GAA CCG TAC TTC AGC TAC TCT GGT GCC TTC AAG TGT CTG AGA GAC GGG GCT GGA GAC GTG GCT TTT ATC AGA GAG AGC ACA GTG TTT  
 188 E P Y F S Y S G A F K C L R D G A G D V A F I R E S T V F  
 1000 GAG GAC CTG TCA GAC GAG GCT GAA AGG GAC GAG TAT GAG TTA CTC TGC CCA GAC AAC ACT CGG AAG CCA GTG GAC AAG TTC AAA GAC  
 217 E D L S D E A E R D E Y E L L C P D N T R K P V D K F K D  
 1087 TGC CAT CTG GCC CGG GTC CCT TCT CAT GCC GTT GTG GCA CGA AGT GTG AAT GGC AAG GAG GAT GCC ATC TGG AAT CTT CTC CGC CAG  
 246 C H L A R V P S H A V V A R S V N G K E D A I W N L L R Q  
 1174 GCA CAG GAA AAG TTT GGA AAG GAC AAG TCA CCG AAA TTC CAG CTC TTT GGC TCC CCT AGT GGG CAG AAA GAT CTG CTG TTC AAG GAC  
 275 A Q E K F G K D K S P K F Q L F G S P S G Q K D L L F K D  
 1261 TCT GCC ATT GGG TTT TCG AGG GTG CCC CCG AGG ATA GAT TCT GGG CTG TAC CTT GGC TCC GGC TAC TTC ACT GCC ATC CAG AAC TIG  
 304 S A I G F S R V P P R I D S G L Y L G S G Y F T A I Q N L  
 1348 AGG AAA AGT GAG GAG GAA GTG GCT GCC CGG GTC GTG TGG TGT GCG GTG GGC GAG GAG CTG CGC AAG TGT AAC CAG  
 333 R K S E E V A A R R A R V V C A V G E Q E L R K C N Q

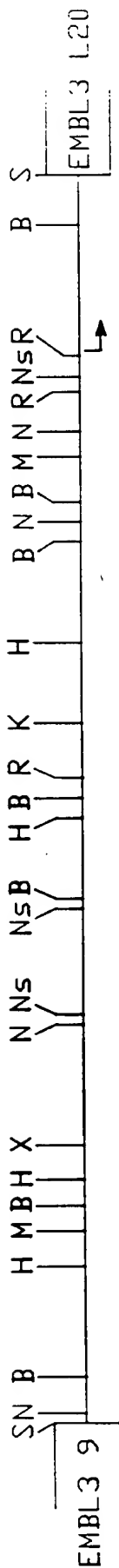
FIG.-2

1435 TGG AGT GGC TTG AGC GAA GGC AGC GTG ACC TGC TCC TCG GCC TCC ACC ACA GAG GAC TGC ATC GCC CTG GTG CTG AAA GGA GAA GCT  
 362 W S G L S E G S V T C S S A S T T E D C I A L V L K G E A  
 1522 GAT GCC ATG AGT TTG GAT GGA TAT GTG TAC ACT GCA TGC AAA TGT GGT TTG GTG CCT GTC CTG GCA GAG AAC TAC AAA TCC CAA  
 391 D A H S L D G G Y Y V Y T A C K C G L V P V L A E N Y K S Q  
 1609 CAA AGC AGT GAC CCT GAT AAC TGT GTG GAT AGA CCT GTG GAA GGA TAT CTT GCT GTG GCG GTG GTT AGG AGA TCA GAC ACT AGC  
 420 Q S S D P D P N C V D R P V E G Y L A V A V R S D T S  
 1696 CTT ACC TGG AAC TCT GTG AAA GGC AAG TCC TGC CAC ACC GCC GTG GAC AGG ACT GCA GGC TGG AAT ATC CCC ATG GGC CTG CTC  
 449 L T W N S V K G K S C N T A V D R T A G W N I P M Q L L  
 1783 TTC AAC CAG ACG GGC TCC TGC AAA TTT GAT GAA TAT TTC AGT CAA AGC TGT GCC CCT GGG TCT GAC CCG AGA TCT AAT CTC TGT GCT  
 478 F N Q T G S C K F D E Y F S Q S C A P G S D P R S N L C A  
 1870 CTG TGT ATT GGC GAC GAG CAG GGT GAG AAT AAG TGC CCC AAC AGC AAC GAG AGA TAC TAC GGC TAC ACT GGG GCT TTC CGG TGC  
 507 L C I G D E Q G E N K C V P N S N E R Y Y G Y T G A F R C  
 1957 CTG GCT GAG AAT GCT GGA GAC GTT GCA TTT GTG AAA GAT GTC ACT GTC TTG CAG AAC ACT GAT GGA AAT AAC AAT GAG GCA TGG GCT  
 536 L A E N A G D V A F V K D V T V L Q N T D G N N E A W A  
 2044 AAG GAT TTG AAC CTG GCA GAC TTT GCG CTG CTG TGC CTC GAT GGC AAA CCG AAG CCT GTG ACT GAC GCT AGA AGC TGC CAT CTT GCC  
 565 K D L K L A D F A L L C L D G K R K P V T E A R S C H L A  
 2131 ATG GCC CCG AAT CAT GCC GTG GTG TCT CCG ATG GAT AAG GTG GAA CCG CTG AAA CAG GTG CTG CTC CAC CAA CAG GCT AAA TTT GGG  
 594 M A P N H A V V S R M D K V E R L K Q V L L H Q Q A K F G  
 2218 AGA AAT GGA TCT GAC TGC CCG GAC AAG TTT TGC TTA TTC CAG TCT GAA ACC AAA AAC CTT CTG TTC AAT GAC AAC ACT GAG TGT CTG  
 623 R N G S D C P D K F C L F Q S E T K N L L F N D N T E C L  
 2305 GCC AGA CTC CAT GGC AAA ACA TAT GAA AAA TAT TTG GGA CCA CAG TAT GTC GCA GGC ATT ACT AAT CTG AAA AAG TGC TCA ACC  
 652 A R L H G K T T Y E K Y L G P Q Y V A G I T N L K K C S T  
 2392 TCC CCC CTC CTG GAA GCC TGT GAA TTC CTC AGG AAG TAA AACCGAAGAA GATGGCCAG CTCGCCAAGA AAGCCTCAGC CATTCACTGC CCCCAGCTCT  
 681 S P L L E A C E F L R K D  
 2491 TCTCCCAGG TGTGTTGGGG CCTTGGCTCC CCTGCTGAAG GTGGGGATTG CCCATCCATC TGCTTACAAT TCCCTGCTGT CGTCTTAGCA AGAAGTAAAA  
 2591 TGAGAAATTT TGTGATATT CAAAAAAA

FIG.-2



RESTRICTION MAP OF 5' - FLANKING REGION OF  $\alpha$ S1 CASEIN GENE



S - SalI

B - BstII

R - EcoRI

H - HindIII

M - SmaI

N - NcoI

Ns - NsiI

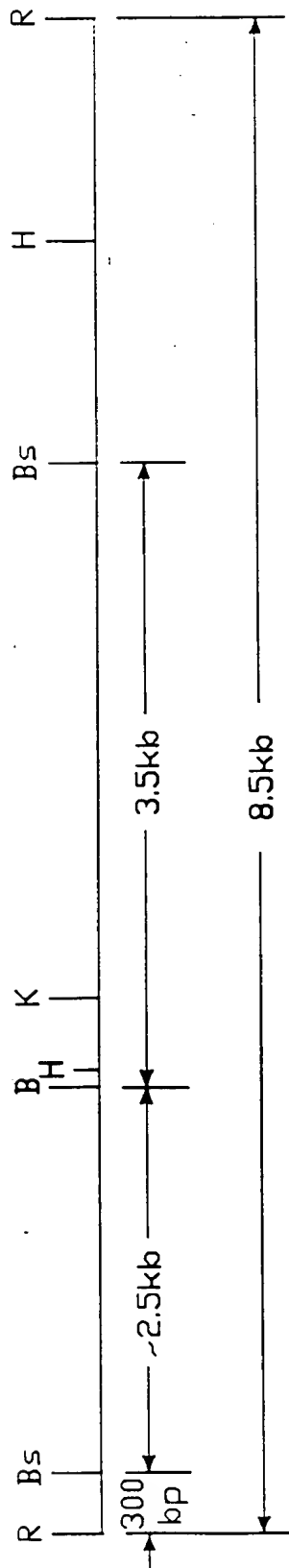
X - XhoI

K - KpnI

1Kb

FIG.-3

RESTRICTION MAP OF 3' FLANKING  
REGION OF  $\alpha$ S1 CASEIN GENE



R - EcoRI

Bs - Bst EII

B - BamHI

H - HindIII

K - KpnI

1kb

FIG.-4

08/476798

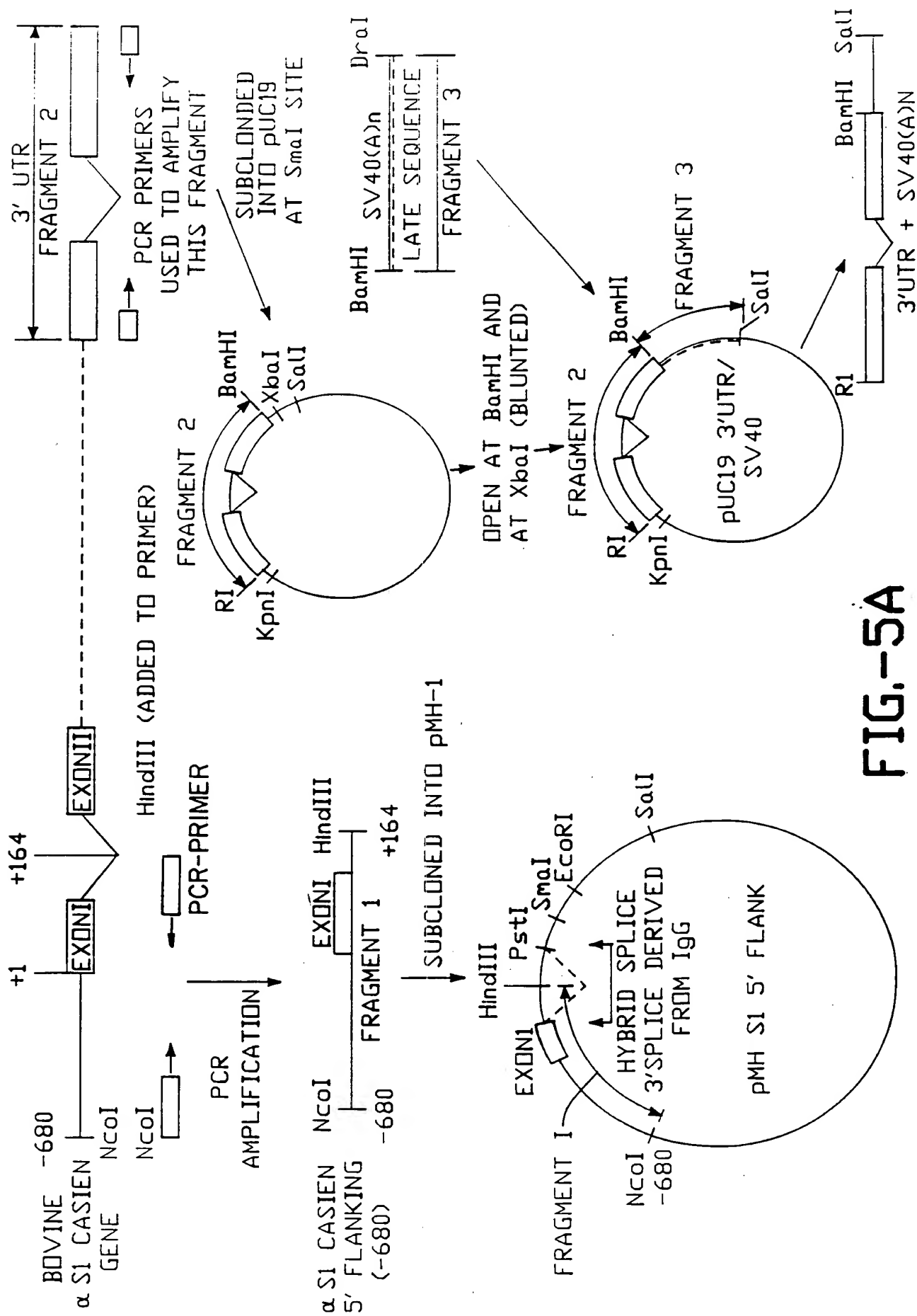


FIG.-5A

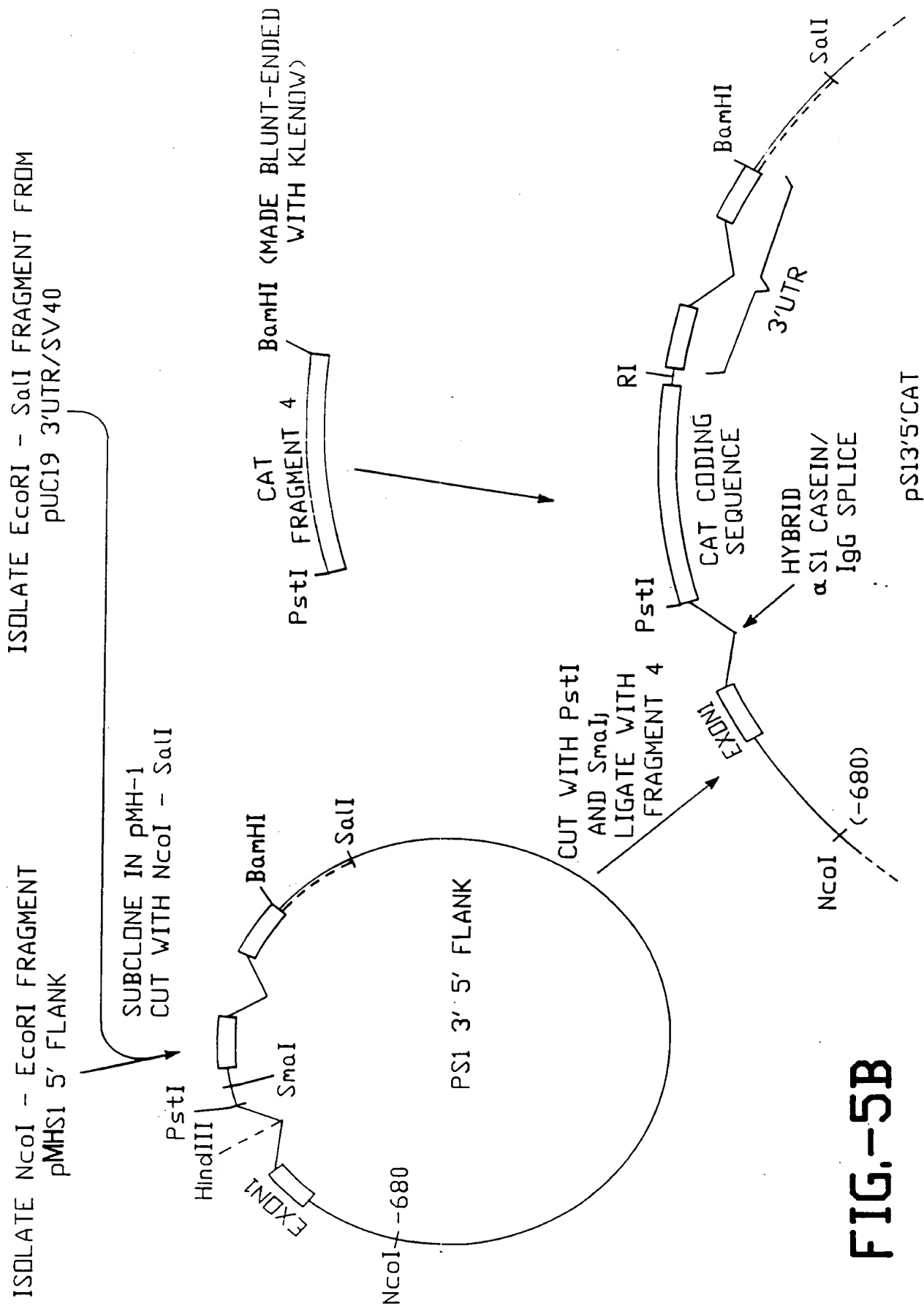
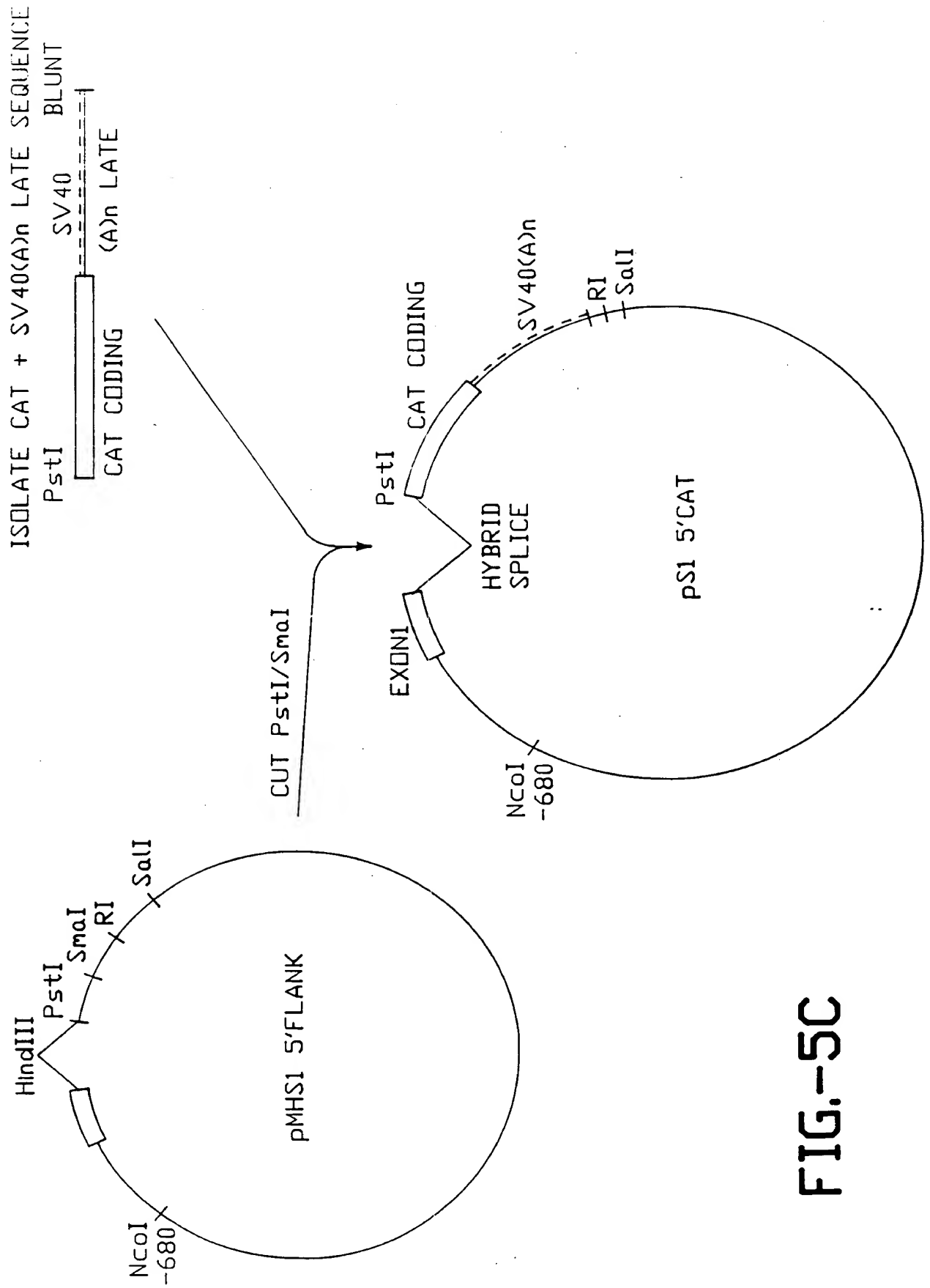


FIG.-5B



NAME pMH-1

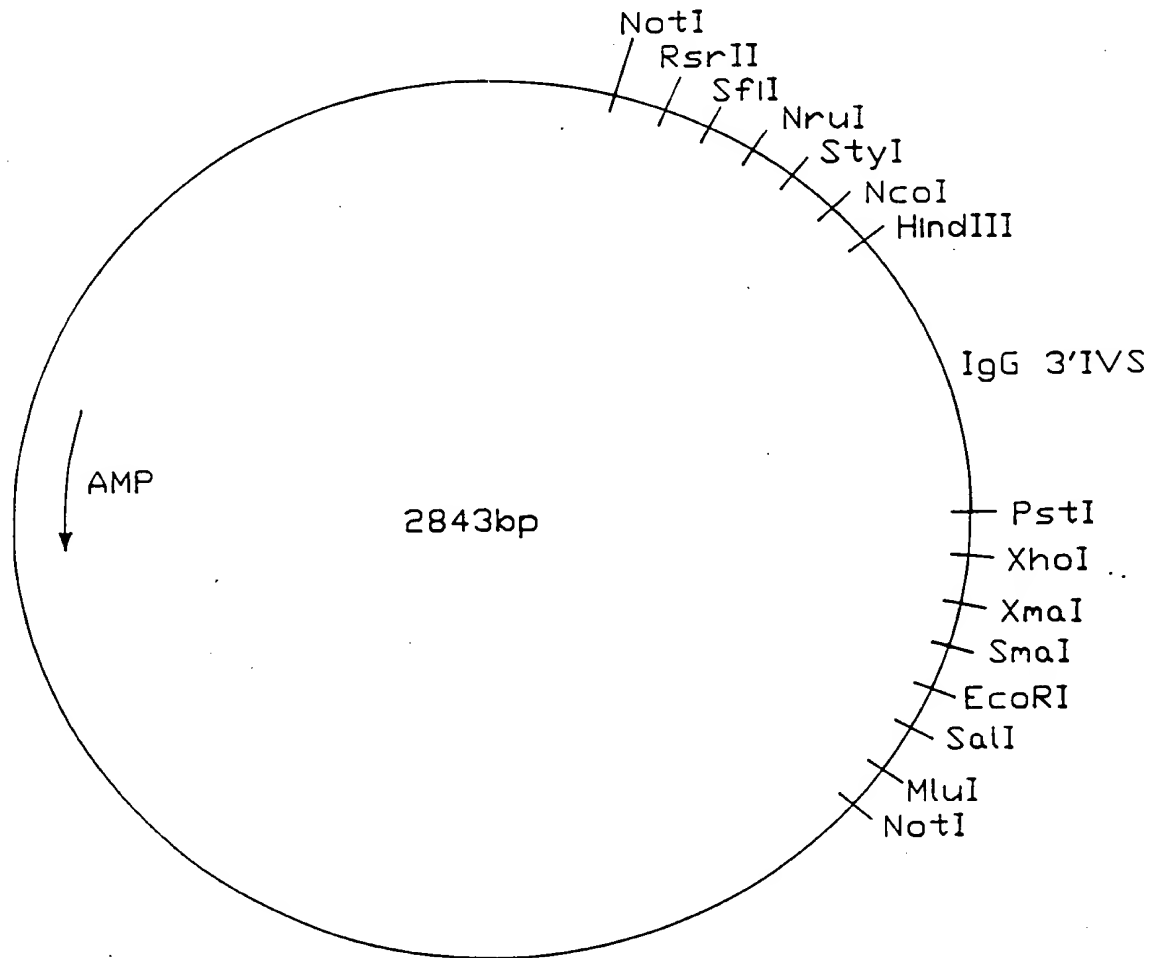


FIG.-6

Diagram of the pMH3'E10 plasmid. The plasmid is a circle with a thick arc representing the  $\alpha$ S1 3' fragment. Restriction sites are marked with tick marks and labels: EcoRI (at the top of the fragment), PstI, HindIII, NcoI, NotI (on the left side), and SalI and NotI (on the right side). The text "pMH3'E10" is in the center.

— PLASMID pMH3'UTR<sub>h</sub>LF2 LINKER

Diagram of the pMH3'UTR construct. A circle represents the plasmid, with 'pMH3'UTR' written inside. A line with a cross indicates the EcoRI restriction site.

Diagram illustrating the structure of the PMH3'UTRhlF2 LINKER construct. The construct is a linear DNA molecule with the following features:

- Restriction Sites:** PstI, EcoRI, KpnI, BamHI, SalI, HindIII, NcoI, and NotI.
- Functional Elements:** 3'UTR  $\alpha S1$  CASEIN, LATE SIGNAL, and SV40(A)<sub>n</sub>.

PMH3'UTR<sub>h</sub>LF2 LINKER

**FIG.-7A**

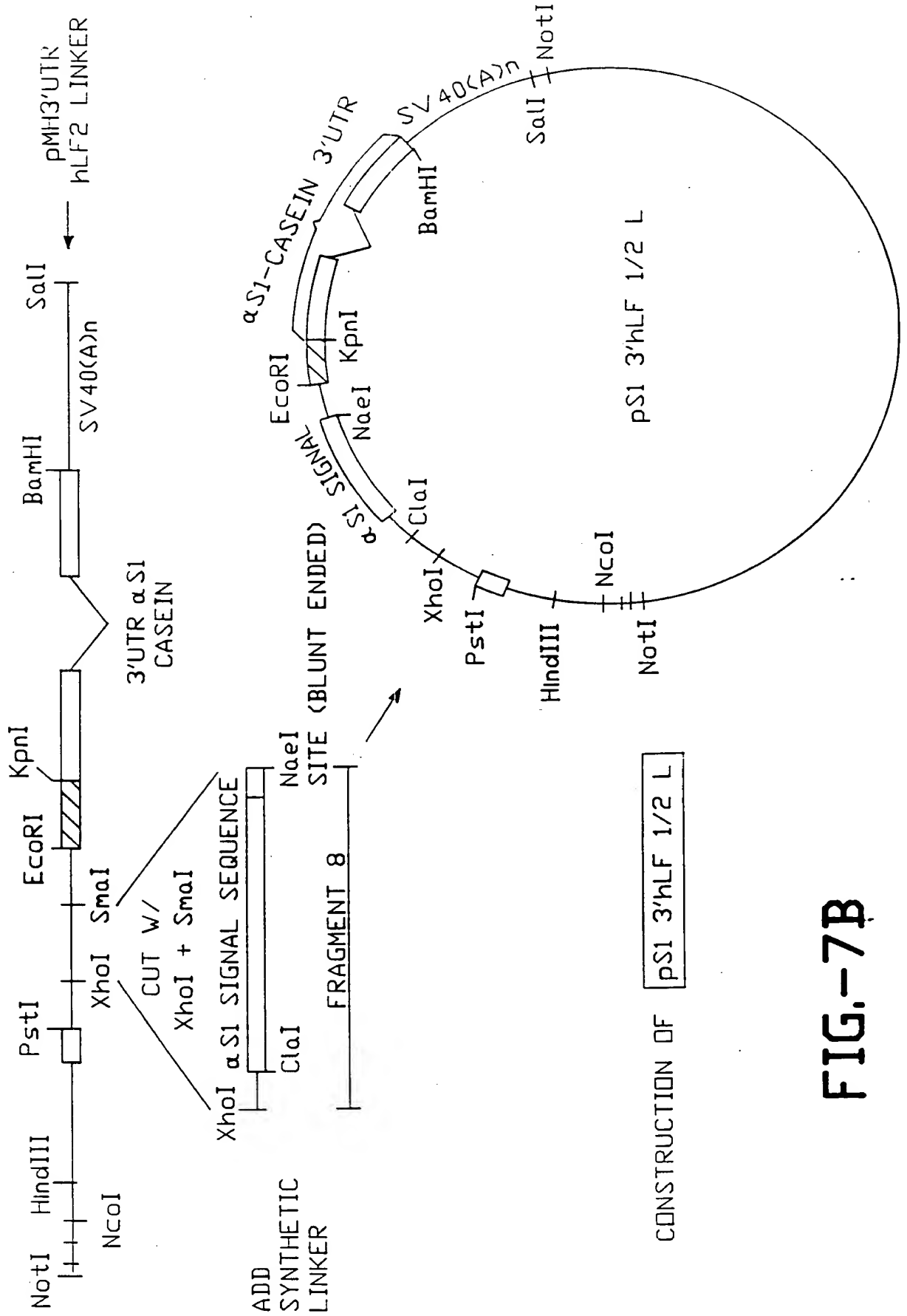


FIG.-7B



# CONSTRUCTION OF pS1 3'UTRhLF

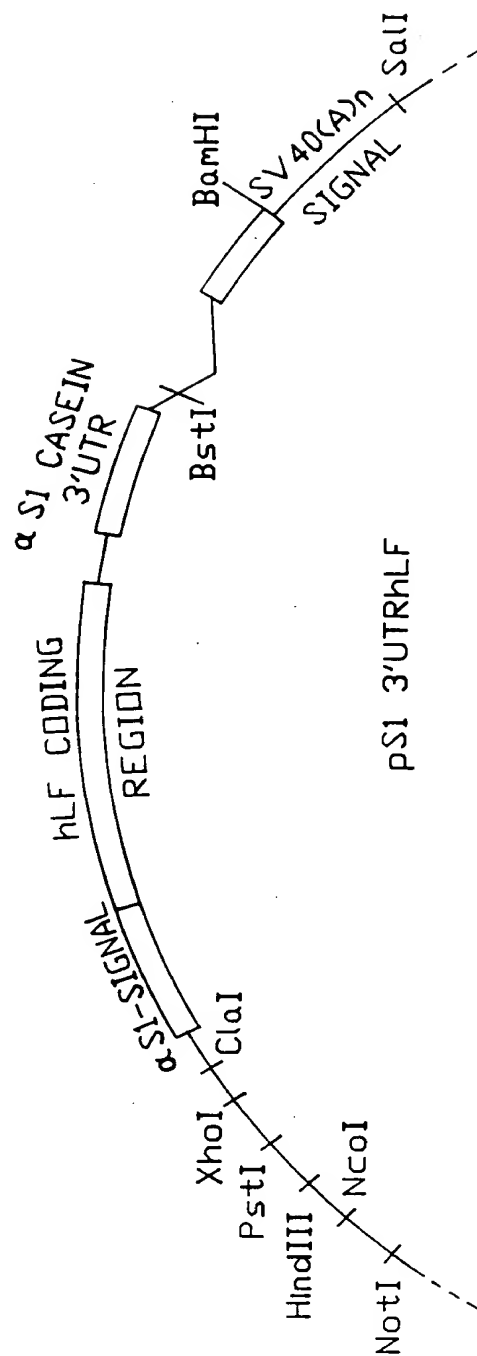
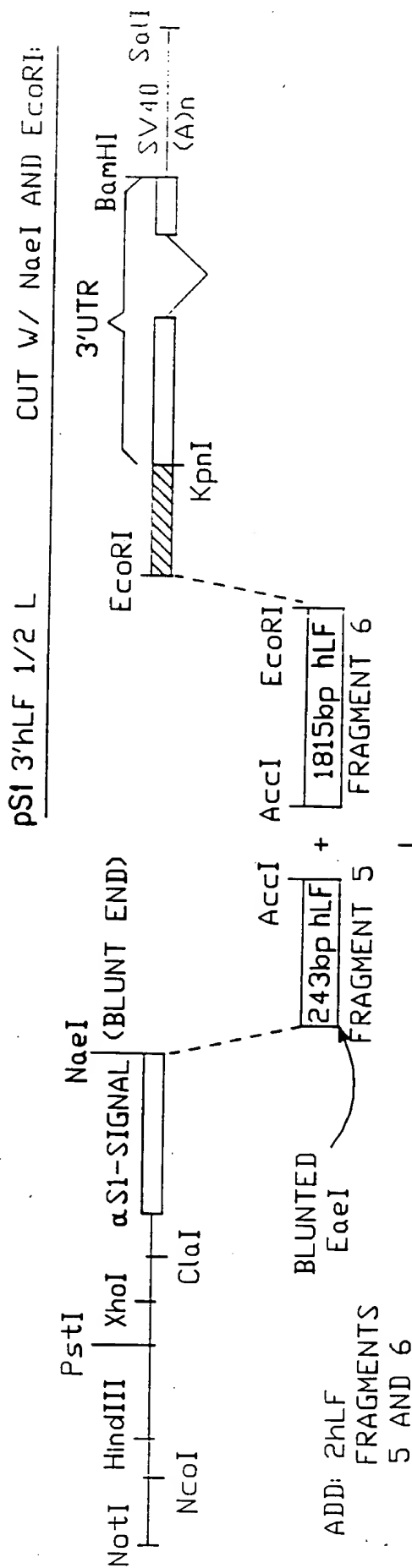


FIG.-7C

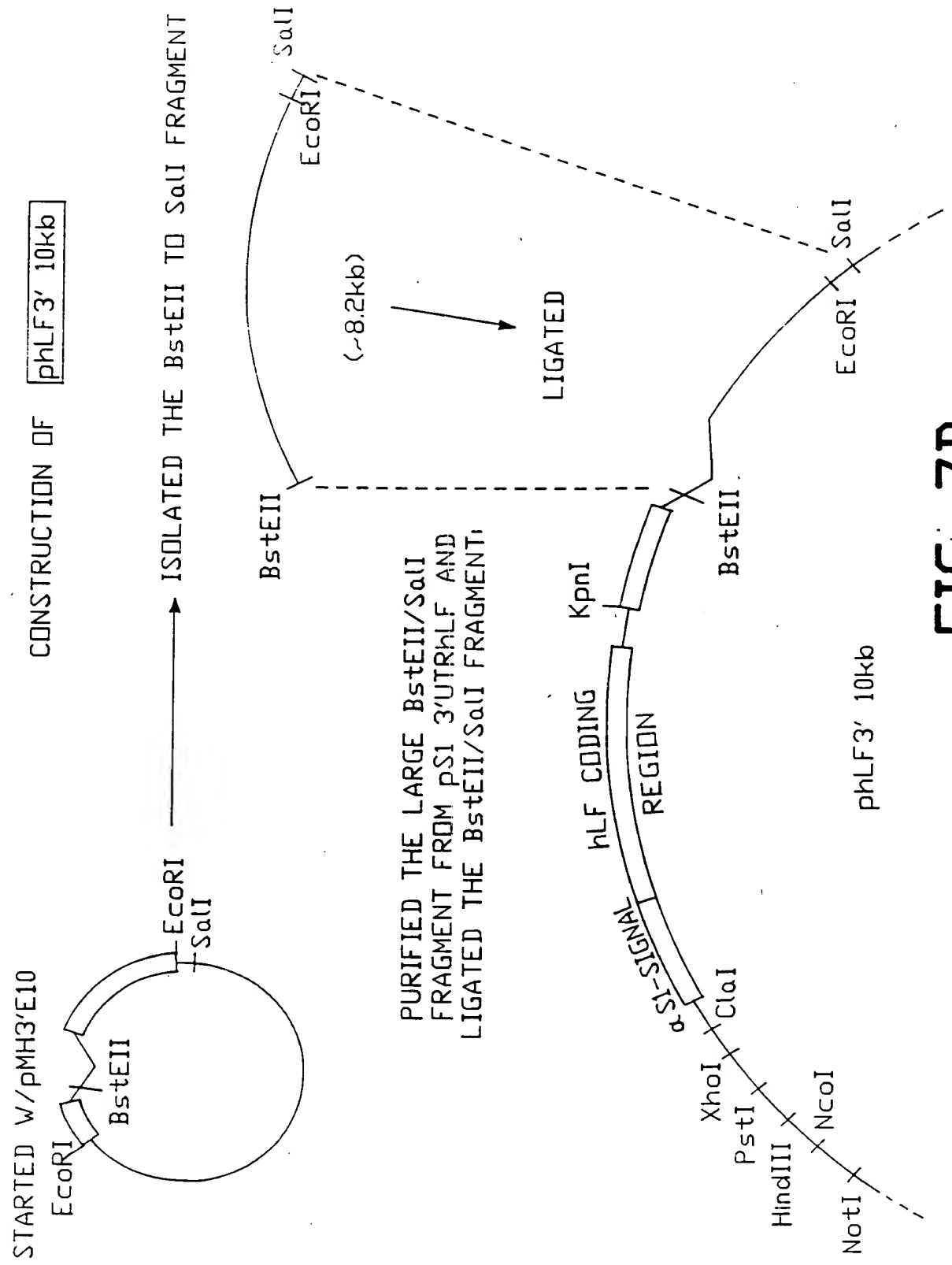
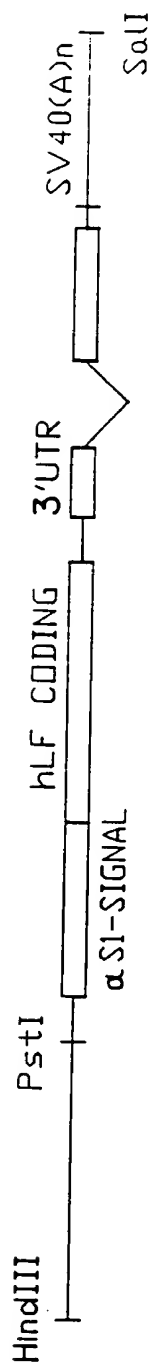


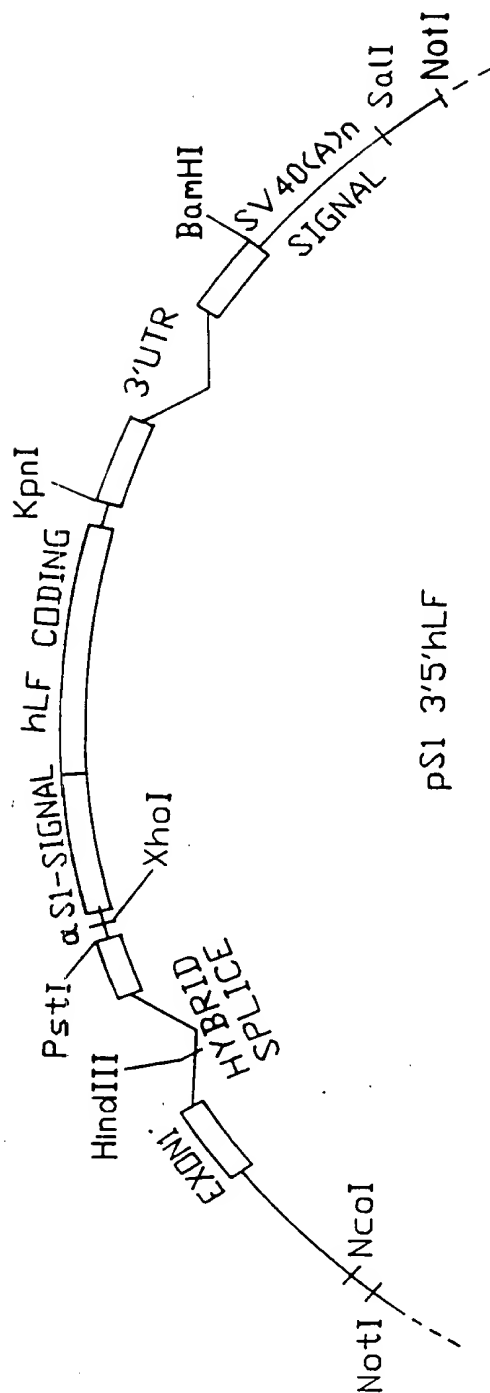
FIG:-7D

CONSTRUCTION OF **pS1 3'5'hLF**

STARTED W/pS1 3'UTR<sub>hLF</sub>. CUT W/HindIII AND SalI AND PURIFIED THIS FRAGMENT CONTAINING THE  $\alpha$ S1-CASEIN SIGNAL SEQUENCE, hLF CODING REGION,  $\alpha$ S1UTR AND SV40(A)<sub>n</sub>.

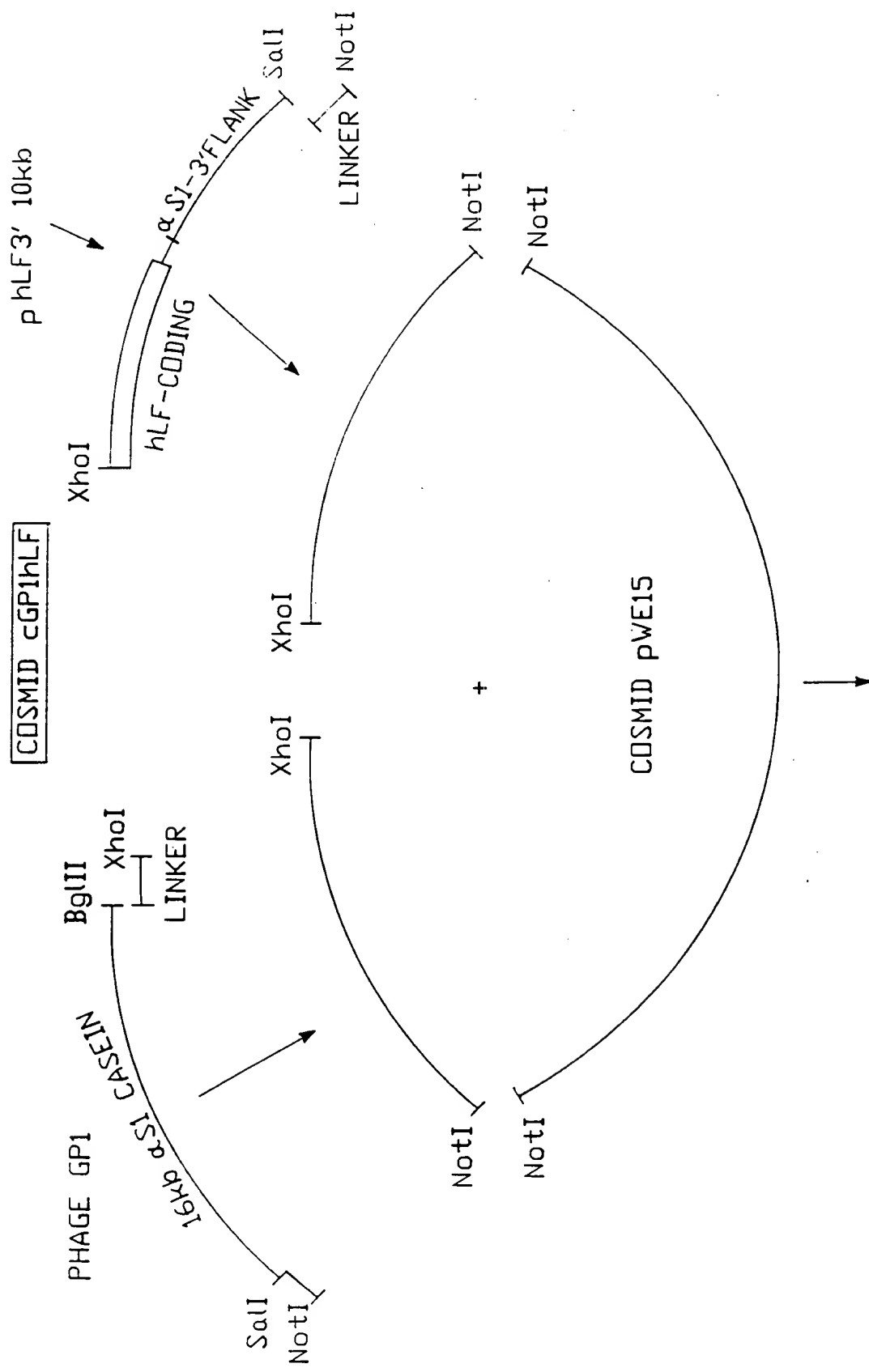


SUBCLONED INTO HindIII/SalI CUT PMHS1 5' FLANK TO YIELD:



NOTE: **pS1 5'hLF** WAS MADE BY CUTTING **pS1 3'5'hLF** W/KpnI AND BamHI, FOLLOWED BY BLUNTING THE ENDS AND RELIGATING. THIS ELIMINATES THE SPLICED 3'UTR REGION.

**FIG.-7E**



3 WAY LIGATION. THE DNA FROM THIS COSMID IS PREPARED BY CUTTING WITH *NotI* AND PURIFYING THE EXPRESSION SEQUENCE PRIOR TO MICROINJECTION.

FIG.-7F

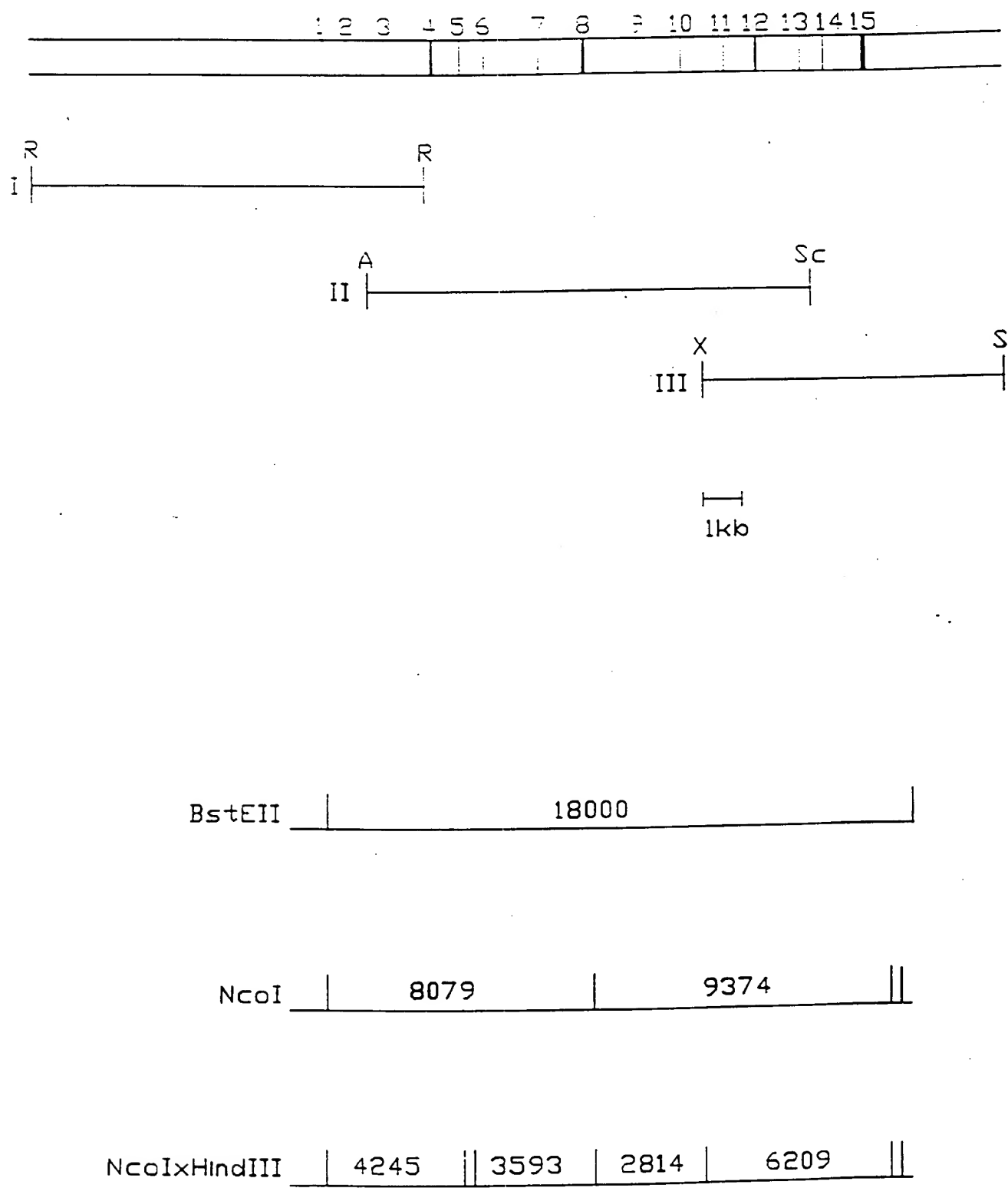
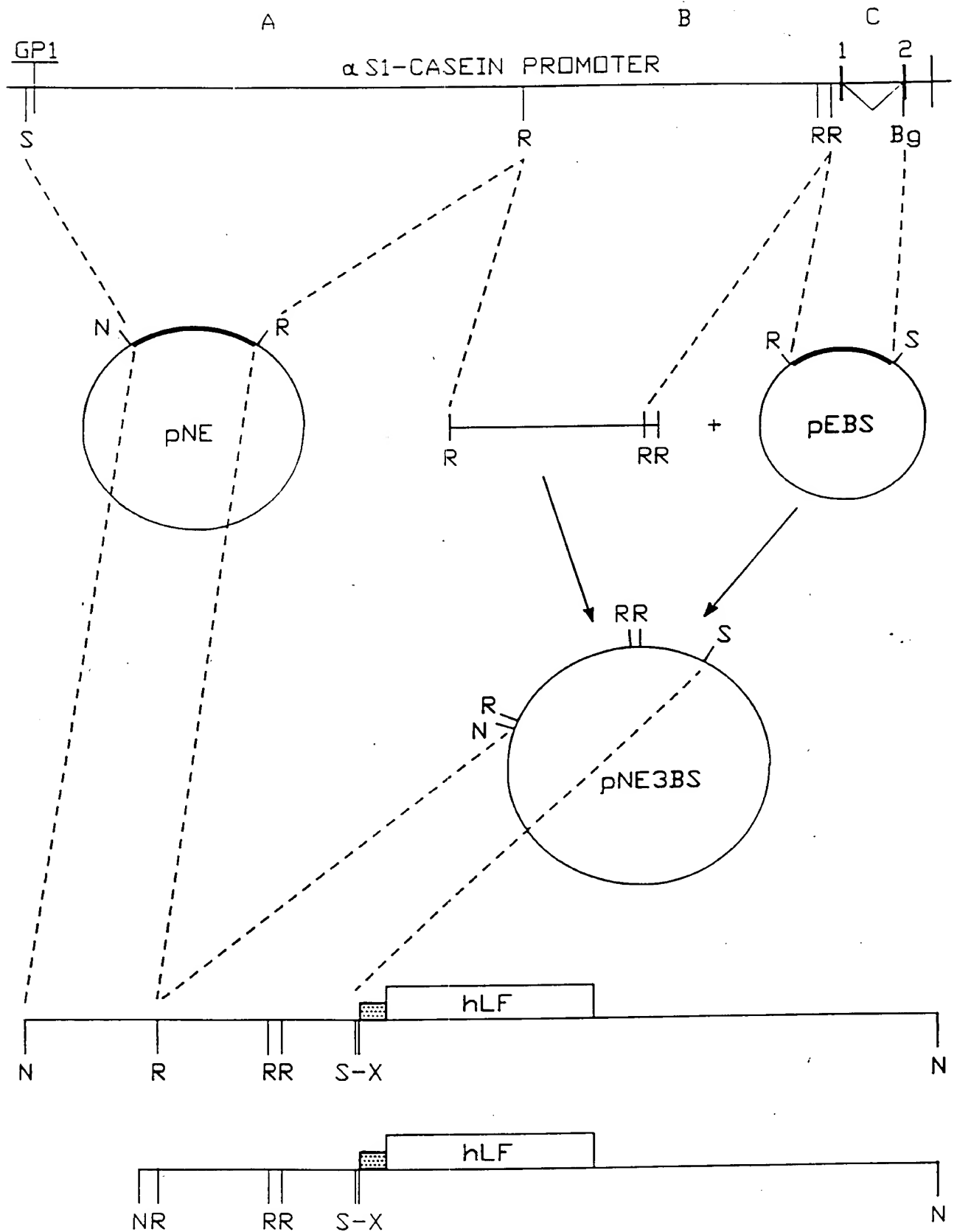


FIG.-8



S=SalI R=EcoRI Bg=BglII N=NotI X=XhoI

FIG.-9

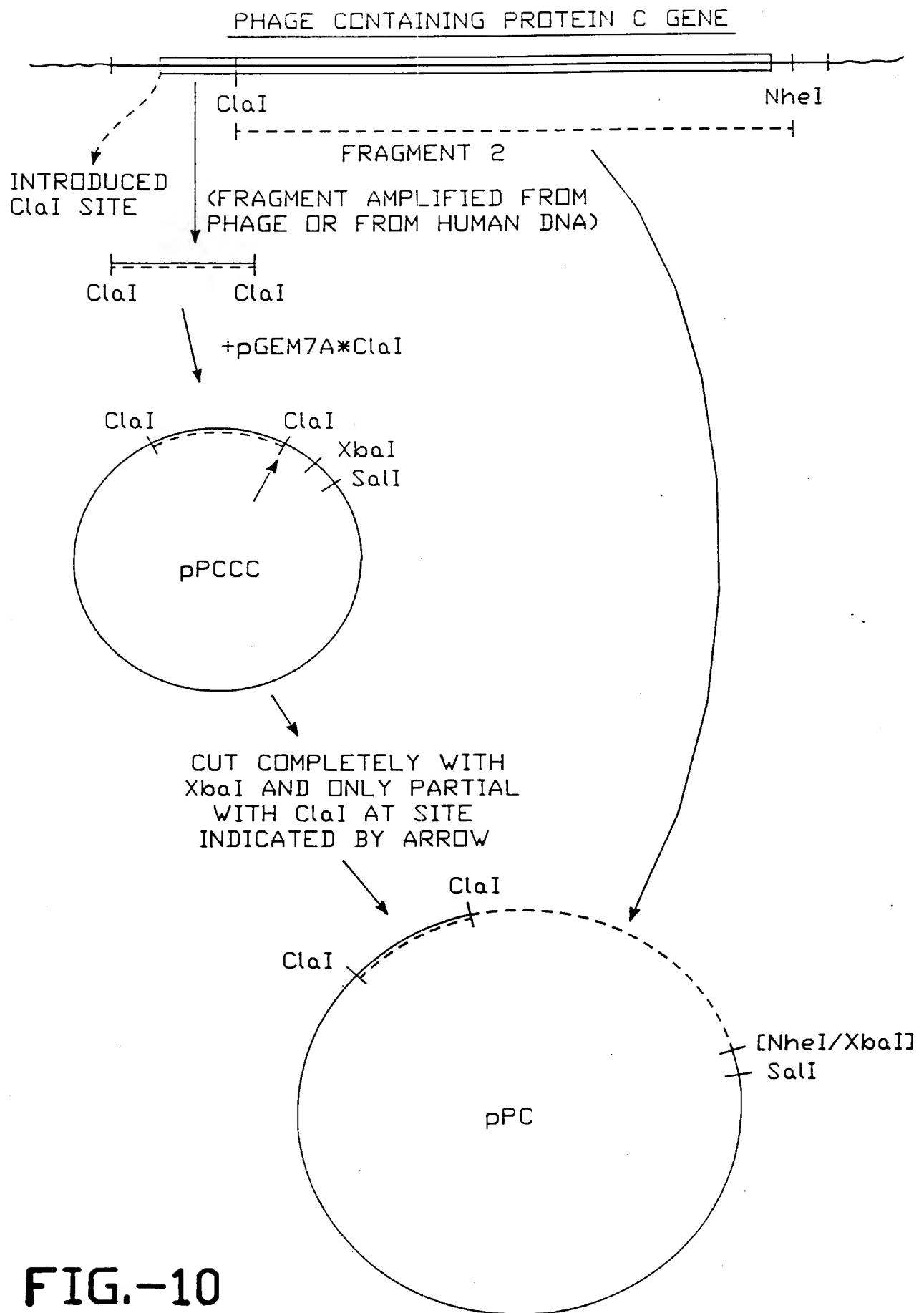


FIG.-10

5'- ATCACCTTGA TCATCAACCC AGCTTGCTGC TTCTTCCCAG  
TCTTGGGTTC AAG gtattatgta tacatataac aaaatttcta tgattttcct ctgtctcacc ..  
tttcattctt cactaatacg cagttgtaac ttttctatgt gattgcaagt attggtactt tctatgata  
tactgttagc aagcttgagg tgtggcaggc ttgagatctg gccatacact tgagtgacaa tgacatccac  
tttgctttc tctccacag GTGTCCACTC CCAGGTCCAA CTGCAG -3'

FIG.-11



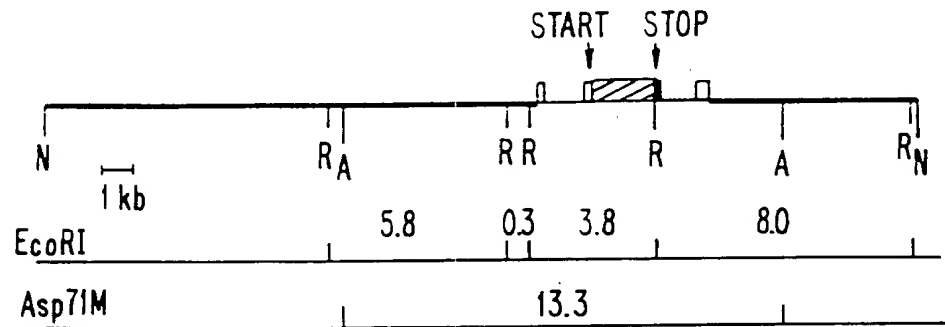


FIG. 12A.

COSMIDS CONTAINING ENTIRE hLF GENE (NOT DRAWN TO SCALE)

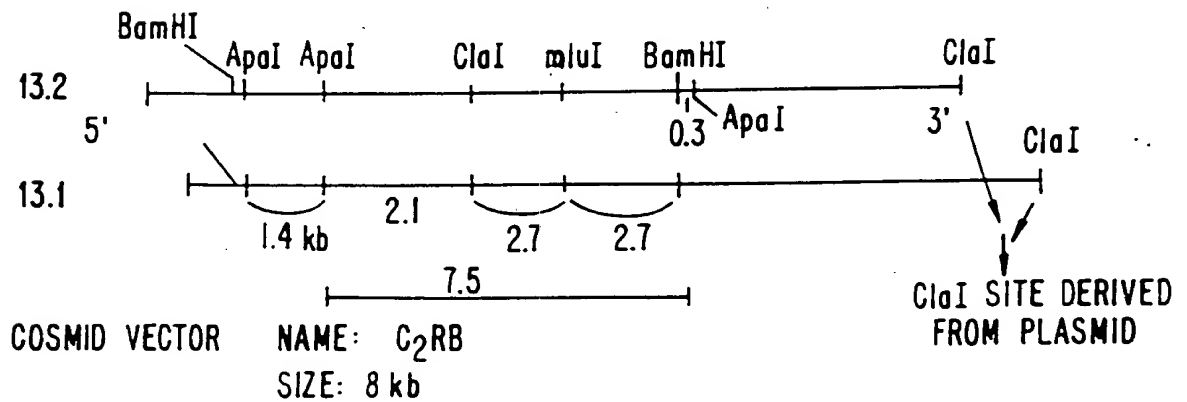


FIG. 13.

9 kb Bam HI hLF FRAGMENT IN pUC19

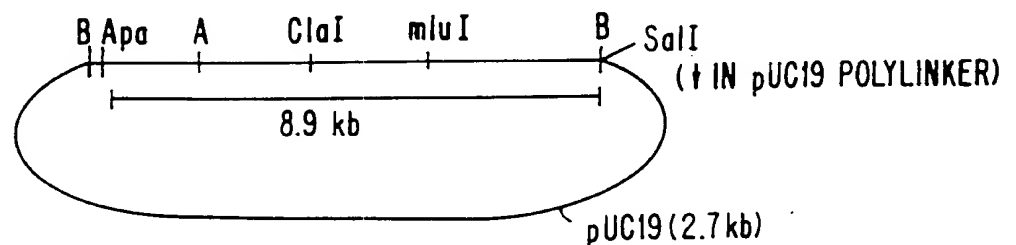


FIG. 14.

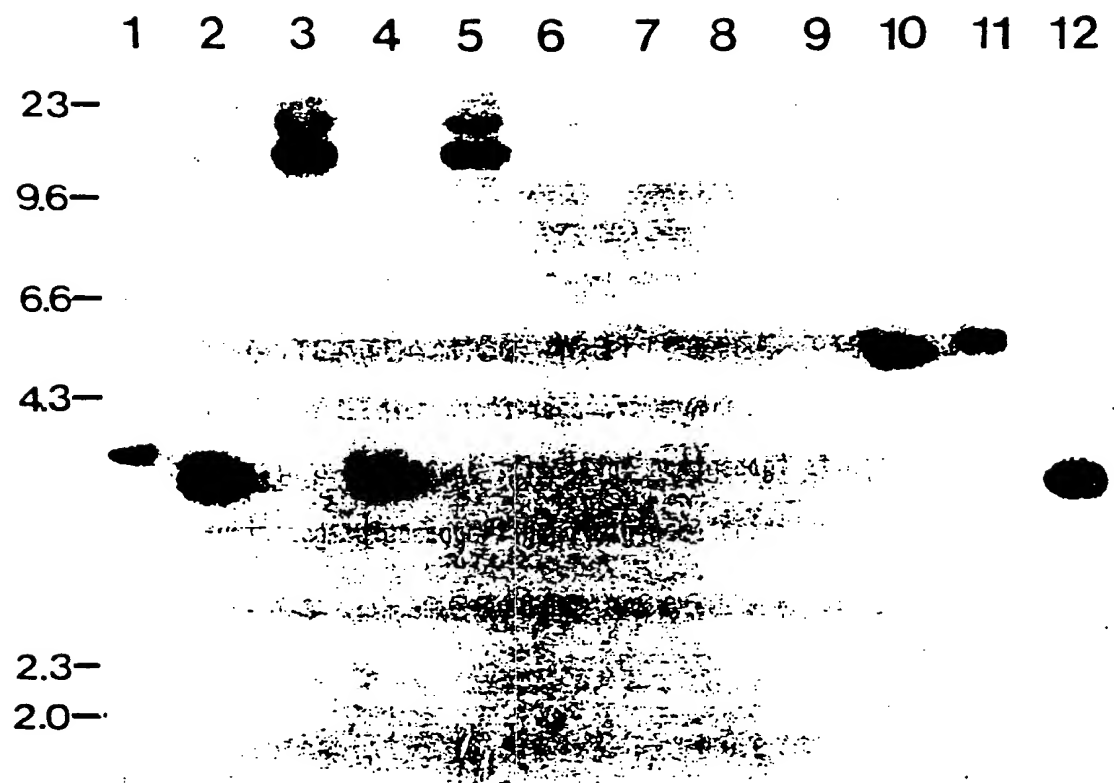
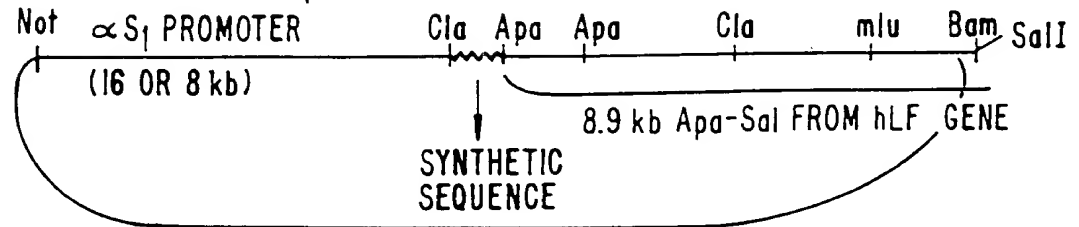


FIG. 12B.

LIGATION PRODUCT OF p16kbCS AND SYNTHETIC SEQ. (Cla-Apa)+ hLF FRAGMENT  
(p8kbCS)



CLONING VECTOR: pkUN (4 kb)

CONSTRUCT NAME: 8 hLF gen 9k, OR 16 hLF gen.9k

FIG. 15A.

STRUCTURE OF ClaI-ApaI SYNTHETIC SEQUENCE

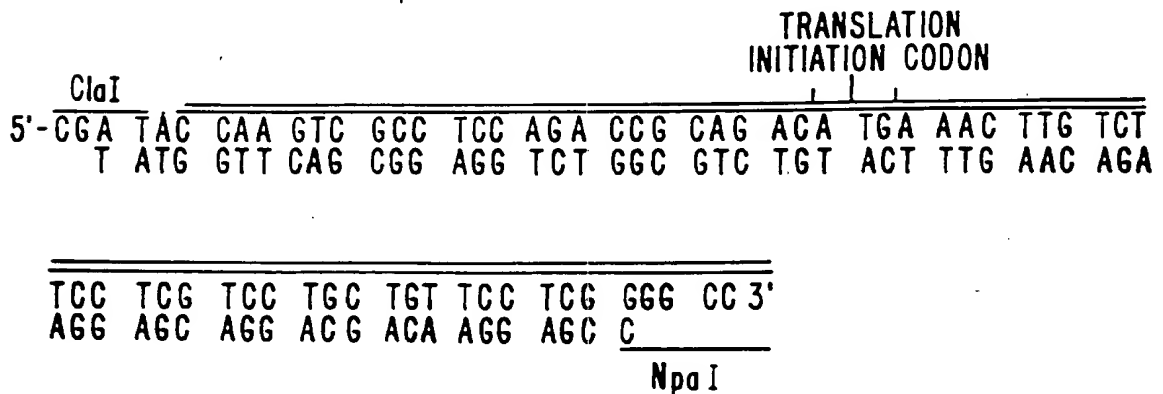
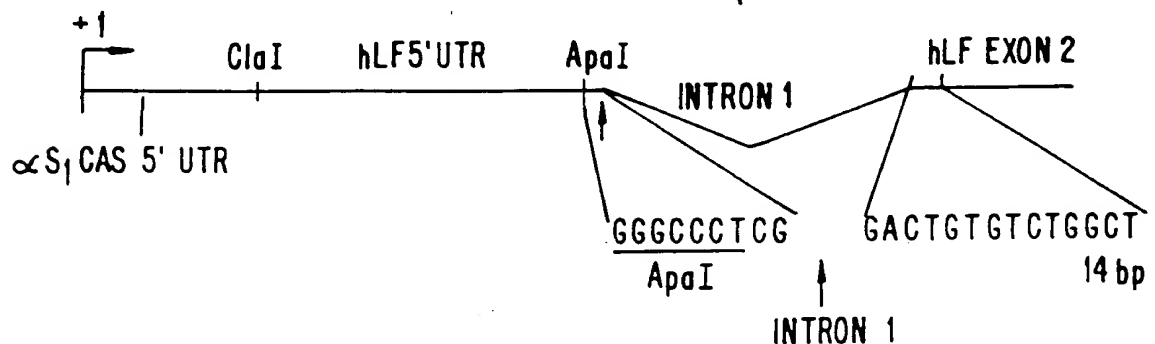


FIG. 15B.

+1: TRANSCRIPTION INITIATION SITE OF BOVINE  $\alpha$ S<sub>1</sub>-CASEIN GENE



STRUCTURE OF REGION CONTAINING EXON 1 (HYBRID  $\alpha$ S<sub>1</sub>-CASEIN/hLF EXON) AND PART OF EXON 2 OF THE GENOMIC hLF CONSTRUCTS DEPICTED IN FIGS. 15A THROUGH 17.

FIG. 15C.

## COINJECTION

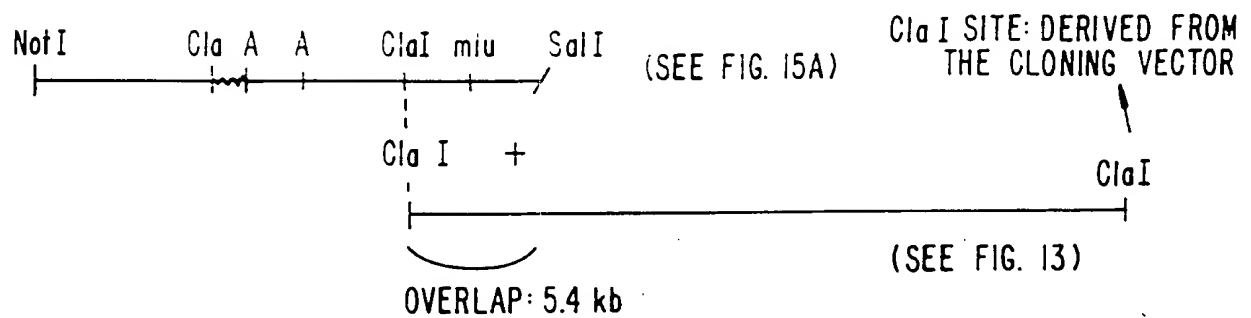
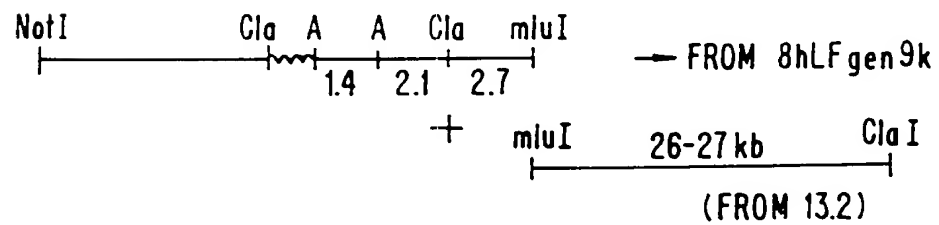


FIG. 16.

## GENERATION OF 8hLF GENE



+

LIGATE INTO Not I CUT COSMID

Cla/Not LINKER:

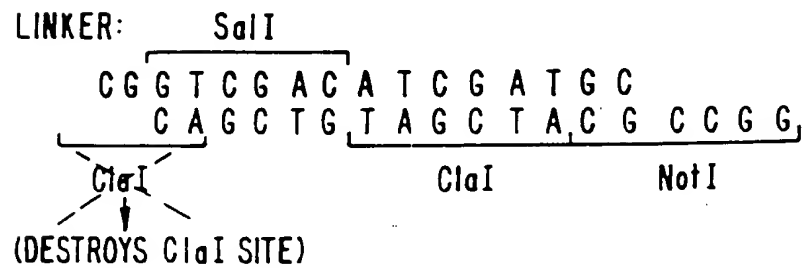


FIG. 17.

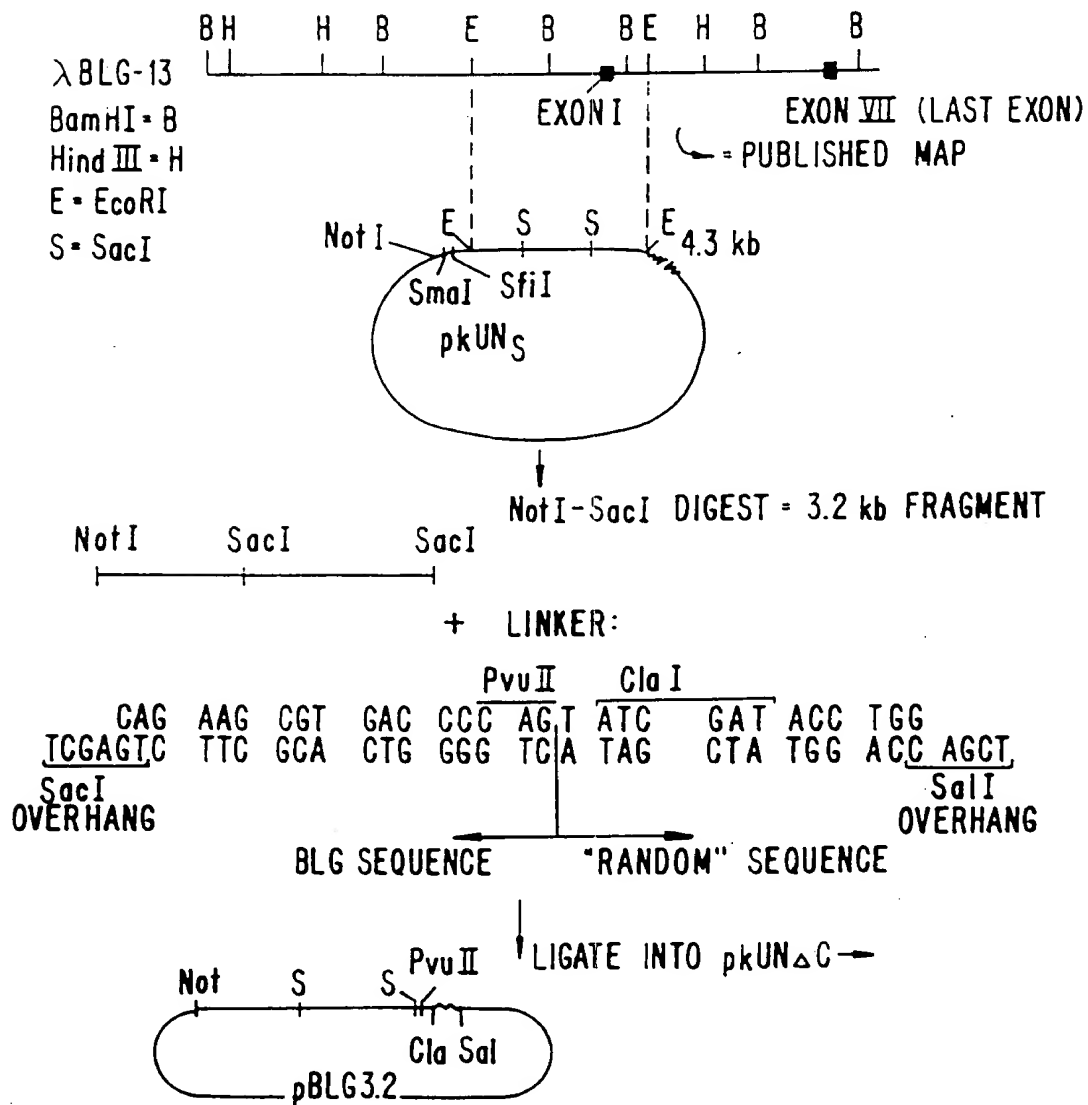


FIG. 18.

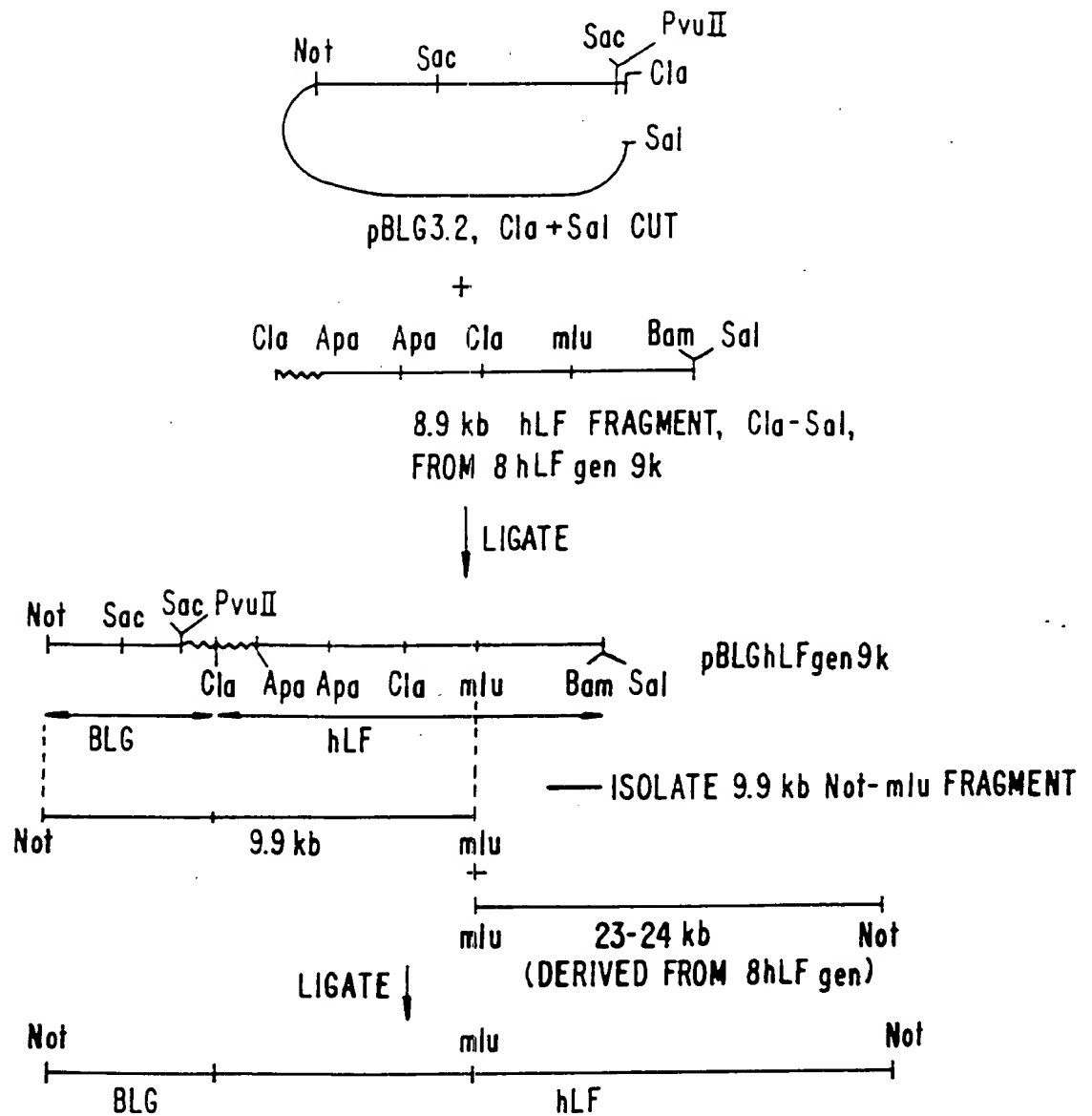


FIG. 19.

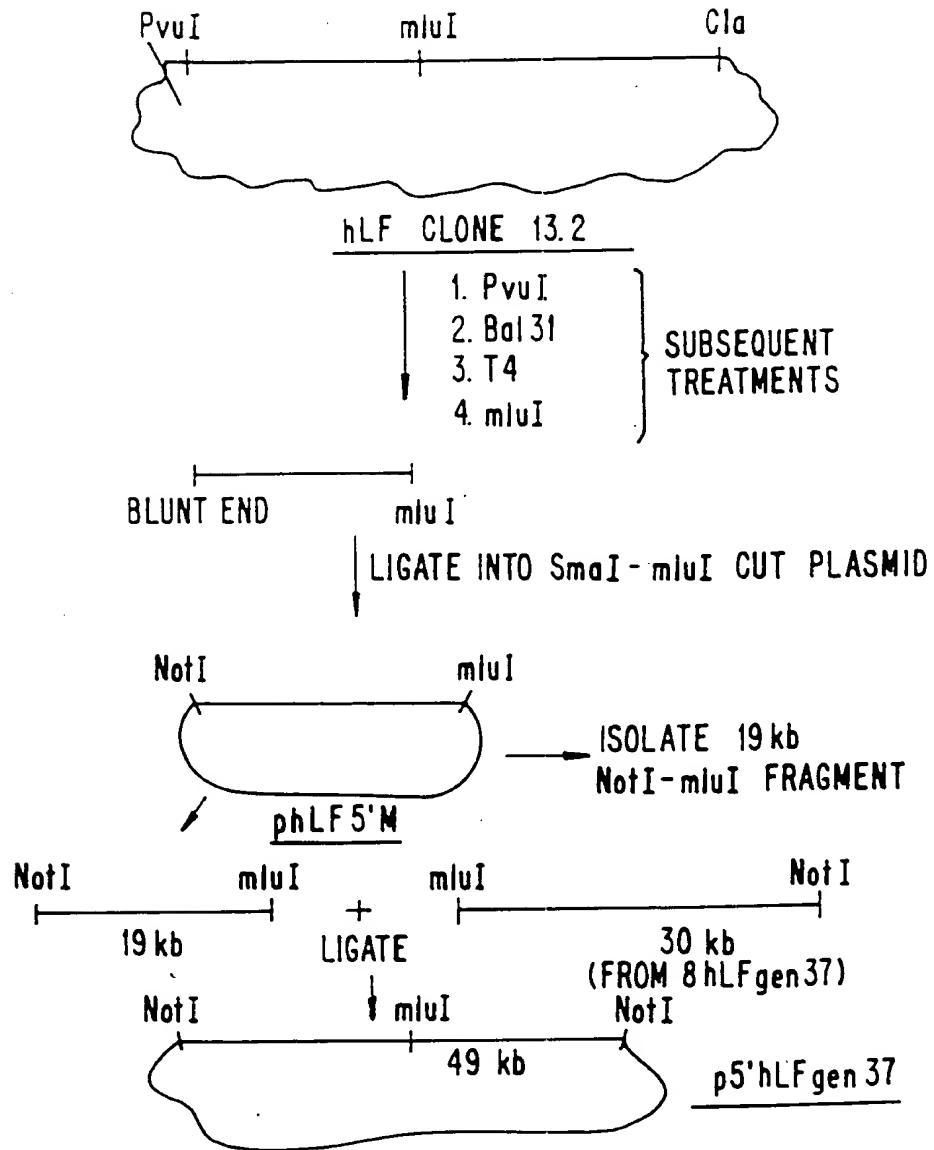


FIG. 20.

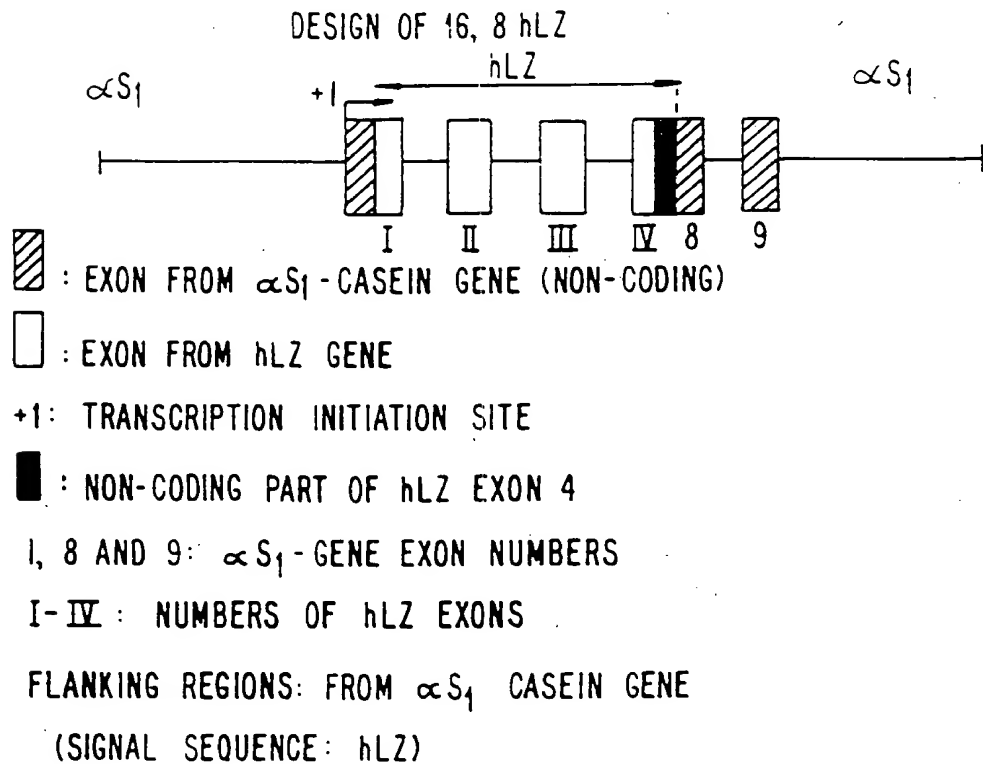


FIG. 21.

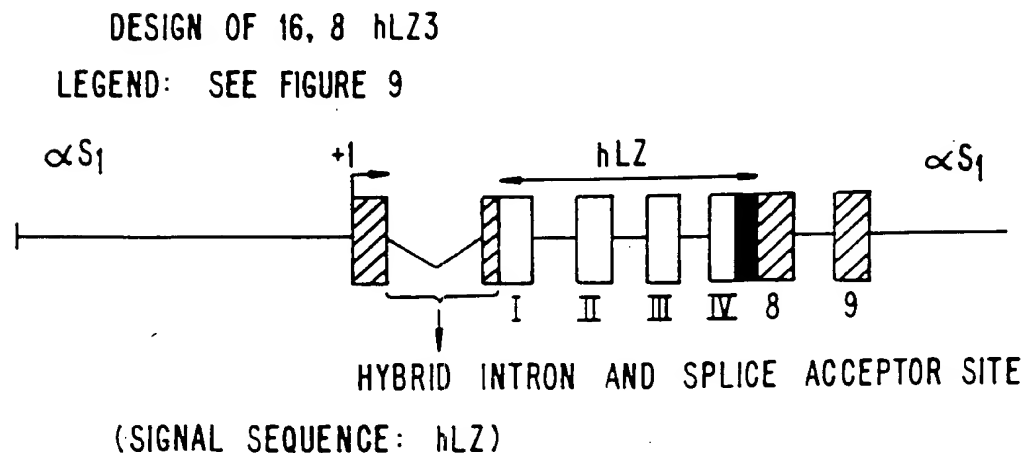


FIG. 22.



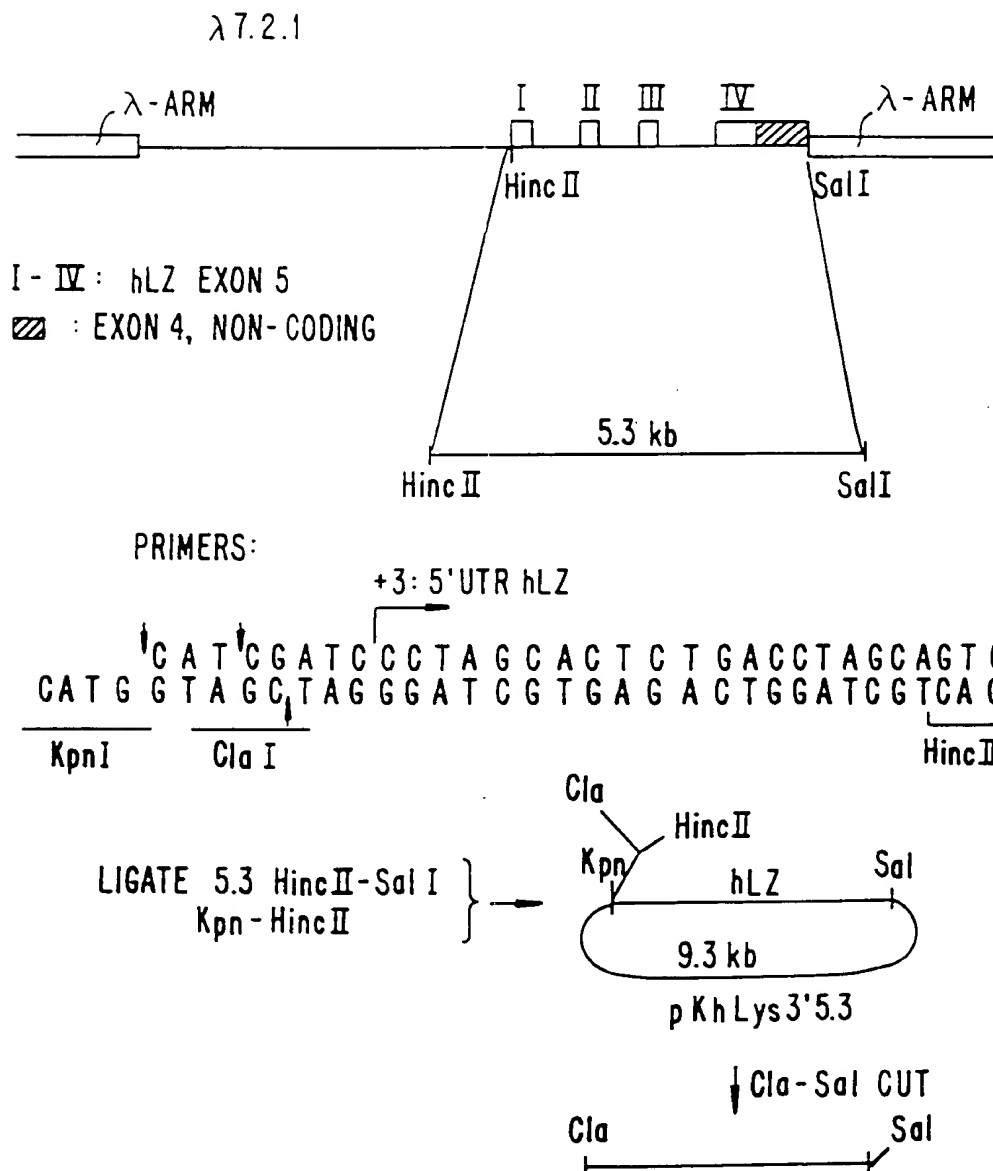


FIG. 23A.

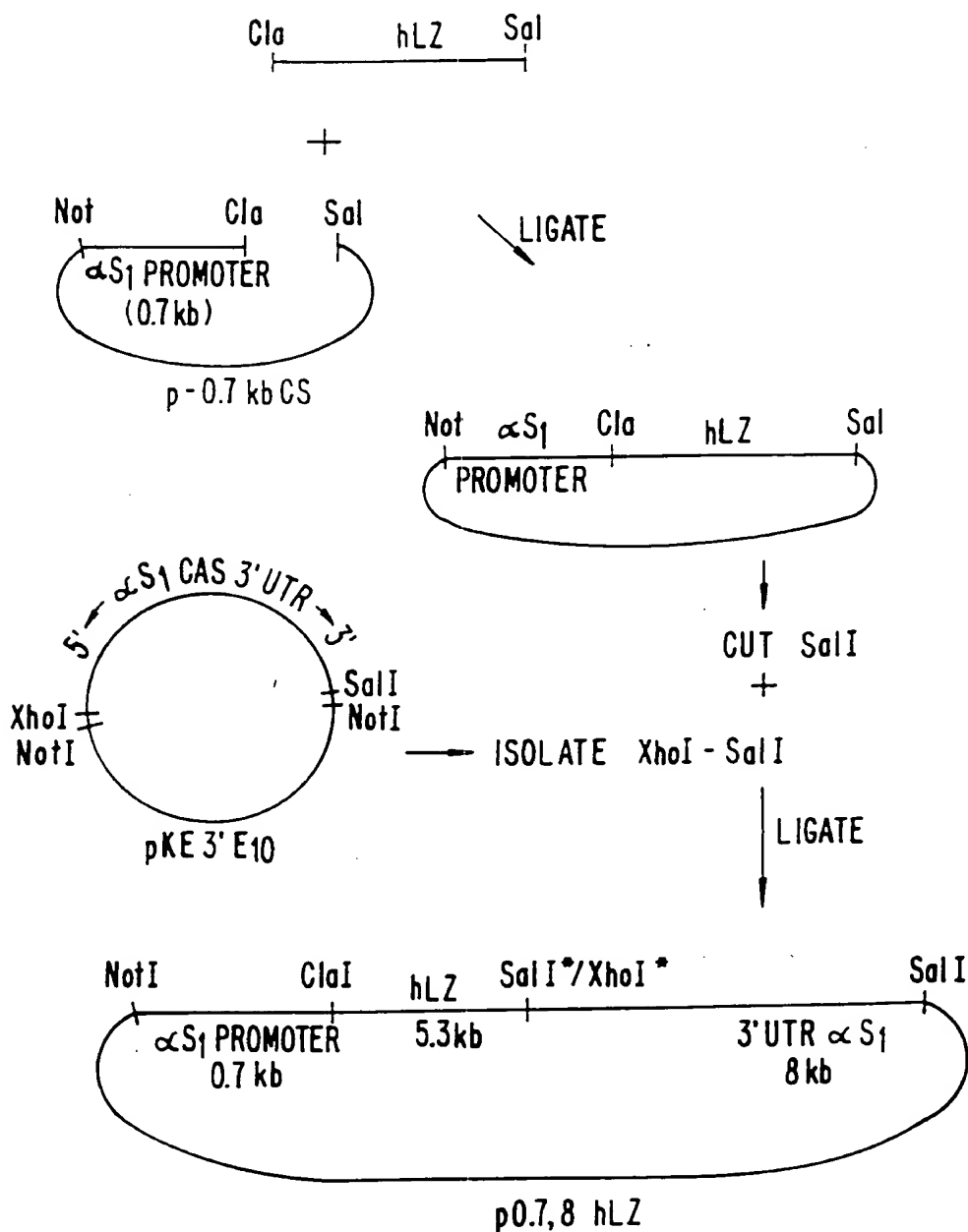


FIG. 23B.

LINKER S<sub>1</sub>/S<sub>2</sub>:SalI<sup>⊗</sup>-NotI-SalI<sup>⊗</sup>

⊗: • DESTROYED SITE

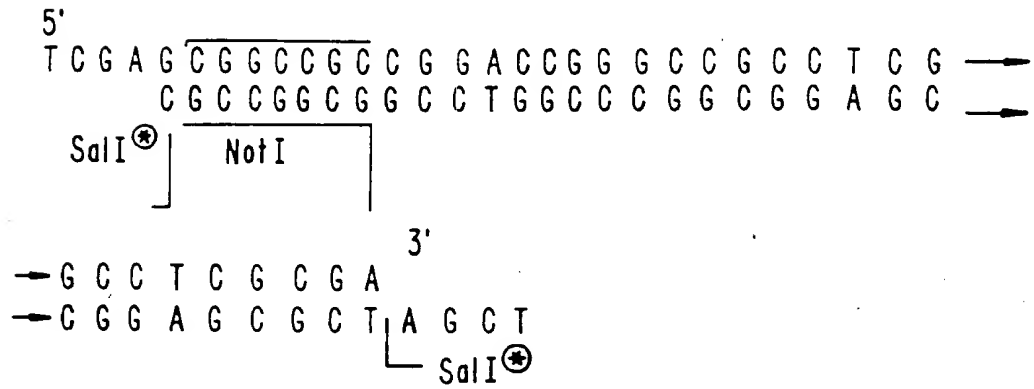


FIG. 23C.

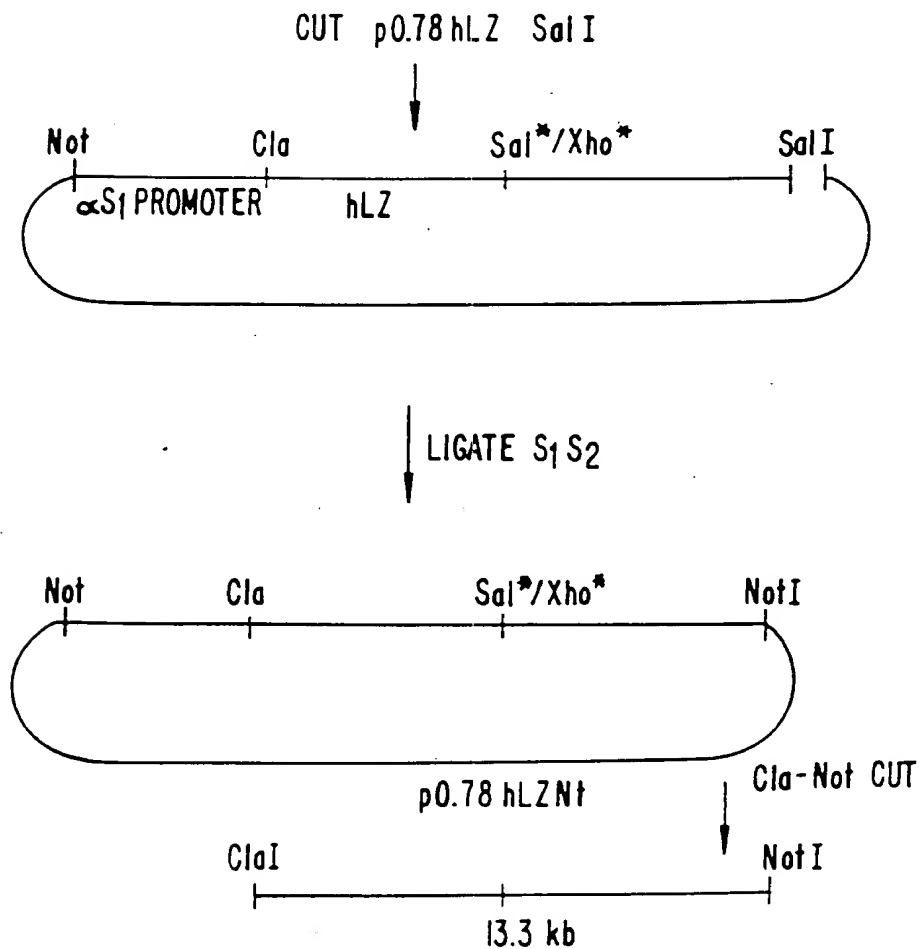
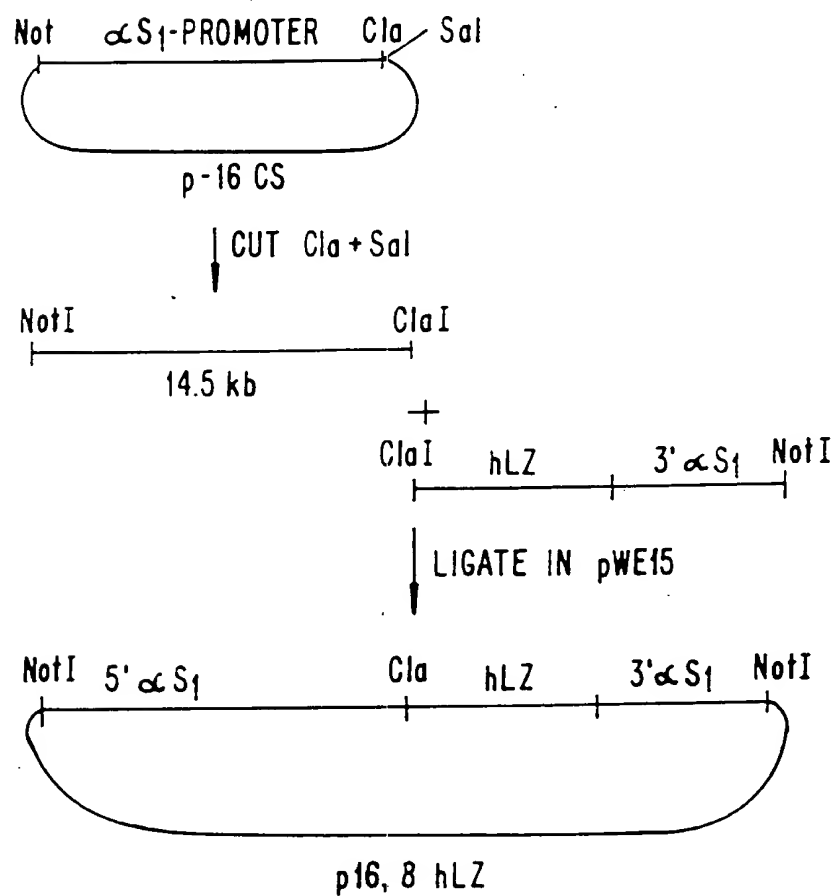


FIG. 23D.

**FIG. 23E.**

BOVINE 2 GGAAGTGCCTGGAGA...TTAAAATGTGAGAGTGGAGT...GGAGGTTG 44  
 |||||  
 SHEEP 84 GGAAGTGTCTCTGGGAGATTTTAAAATGTGAGAGGCGGGAGGTGGGAGGTTG 133  
 |||||  
 45 GGTCTGTAGGCCTTCCCATCCACGTGCCTCAGGGAGCCCTAGTGCTAC 94  
 |||||  
 134 GGGCCTGTGGGCCTGCCCATCCACGTGCCTGCATTAGCCCCAGTGCTGC 183  
 |||||  
 95 TCAGTCATGCCCCCGCAGCAGGGGTCAAGTCACTTTCCCATCCTGGGGGT 144  
 |||||  
 184 TCAGCCGTGCCCCCGCCGCAAGGGTCAAGTCACTTTCCCGTCCT.GGGGT 232  
 |||||  
 145 TATTATGACTGTTGTCATTGTTGTTGCCATTTTTTGCTACCCTAACTGGGC 194  
 |||||  
 233 TATTATGACTCTTGTCATTGCCATTGCCATTTTTTGCTACCCTAACTGGGC 282  
 |||||  
 195 AGCGGGTGCTTGCGAGAGCCCTCGATACTGACCAAGTTCCCCCTCGGAGC 244  
 |||||  
 283 AGCAGGTGCTTGCGAGAGCCCTCGATACCGACCAGG.TCCTCCCTCGGAGC 331  
 |||||  
 245 TCGACCTGAACCCCATGTACCCCTCGCCCCAGCCTGCAGAGGGTGGGTGA 294  
 |||||  
 332 TCGACCTGAACCCCATGTACCCCTTGCCCCAGCCTGCAGAGGGTGGGTGA 381  
 |||||  
 295 CTGCAGAGATCCCTTTACCCAAGGCCACAGTCAATGGTTTGGAGGAGAT 344  
 |||||  
 382 CTGCAGAGATCCCTTCACCCAAGGCCACGGTCAATGGTTTGGAGGAGCT 431  
 |||||  
 345 GGTGCCCAAGGCAGAGGCCACCCCTCCA.GACACACCTGCCCCCAGTGCTG 393  
 |||||  
 432 GGTGCCCAAGGCAGAGGCCACCCCTCCAGGACACACCTGTCCCCAGTGCTG 481  
 |||||  
 394 GCTCTGACCTGTCCTTGTCTAAGAGGGCTGACCCCAAGAGTGTTCCTGGCG 443  
 |||||  
 482 GCTCTGACCTGTCCTTGTCTAAGAGGGCTGACCCCGGAAGTGTTCCTGGCA 531  
 |||||  
 444 CTGGCAGCCAGCCTGGACCCAGAGCCTGGACACCC.CCTGCGCCCCCACT 492  
 |||||  
 532 CTGGCAGCCAGCCTGGACCCAGAGTCCAGACACCCACCTGTGCCCCCGCT 581  
 |||||  
 493 TCTGGGGGCGTACCAGGAACCGTCCAGGCCCAGA..GGGCCTTCCTGCTT 540  
 |||||  
 582 TCTGGGGTC.TACCAGGAACCGTCTAGGCCCAGAGGGGGACTTCCTGCTT 630  
 |||||  
 541 GGCCTCGAATGGAAGAAGGCCTCCTATTGTCTTTCGTAGAGGAAGCAACC 590  
 |||||  
 631 GGCCTTGGATGGAAGAAGGCCTCCTATTGTCC.TCGTAGAGGAAGCCACC 679  
 |||||  
 591 CCAAGGGCCCAAGGATAGGCCAGGGGGGATTCCGGGAACCGCGTGGCT.CC 639  
 |||||  
 680 CCGGGGCGCTGAGGATGAGCCAAAGTGGGATTCCGGGAACCGCGTGGCTGGG 729  
 |||||  
 640 GCGCGGGCCCGGGCTGGCTGGCTGGC..CCTCCTCCTGTATAAGGCCCCG 687  
 |||||  
 730 GGCCAGCCCGGGCTGGCTGGCTGCATGCGCCTCCTGTATAAGGCCCCA 779  
 |||||

FIG. 24

**FIG. 24** [continued]

TRANSLATION START SITE ( $\alpha S_1$  SIGNAL SEQUENCE)

Clal  
CGATAACCATGAACTTCTTATCCTCACCTGTCTTGTGGCTGTTGCTCTTG  
|  
TATT — ETC.

$\alpha S_1$  — hLZ SEQUENCE Bal I  
 — CCAAGGTCTTTGAAAGGTGTGAGTTGC  
 ETC. — AACC

FIG. 25.

## CONSTRUCTION OF 16.8 A hLZ3:

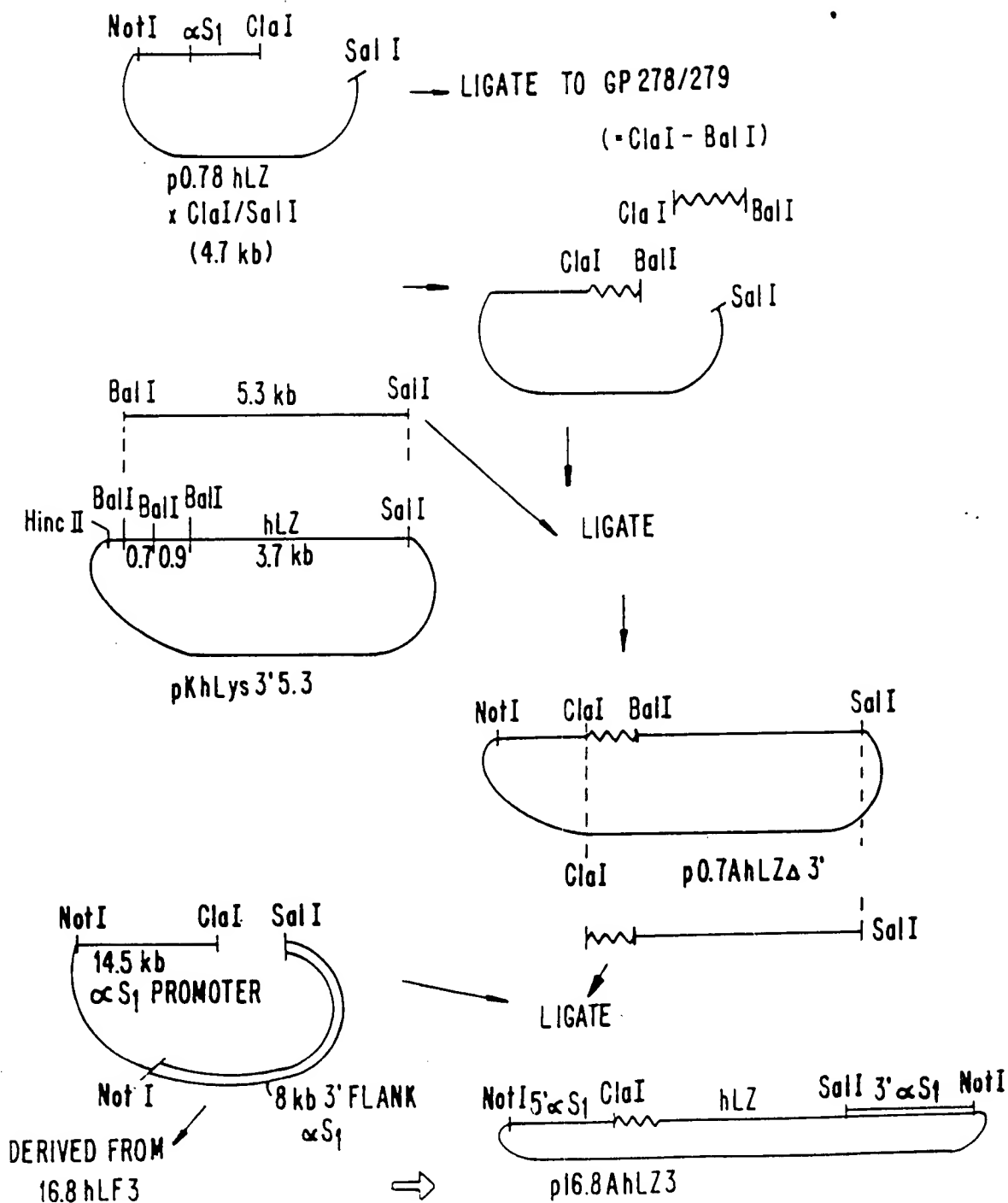


FIG. 26.

CONSTRUCTION OF 16 A hLZ3:

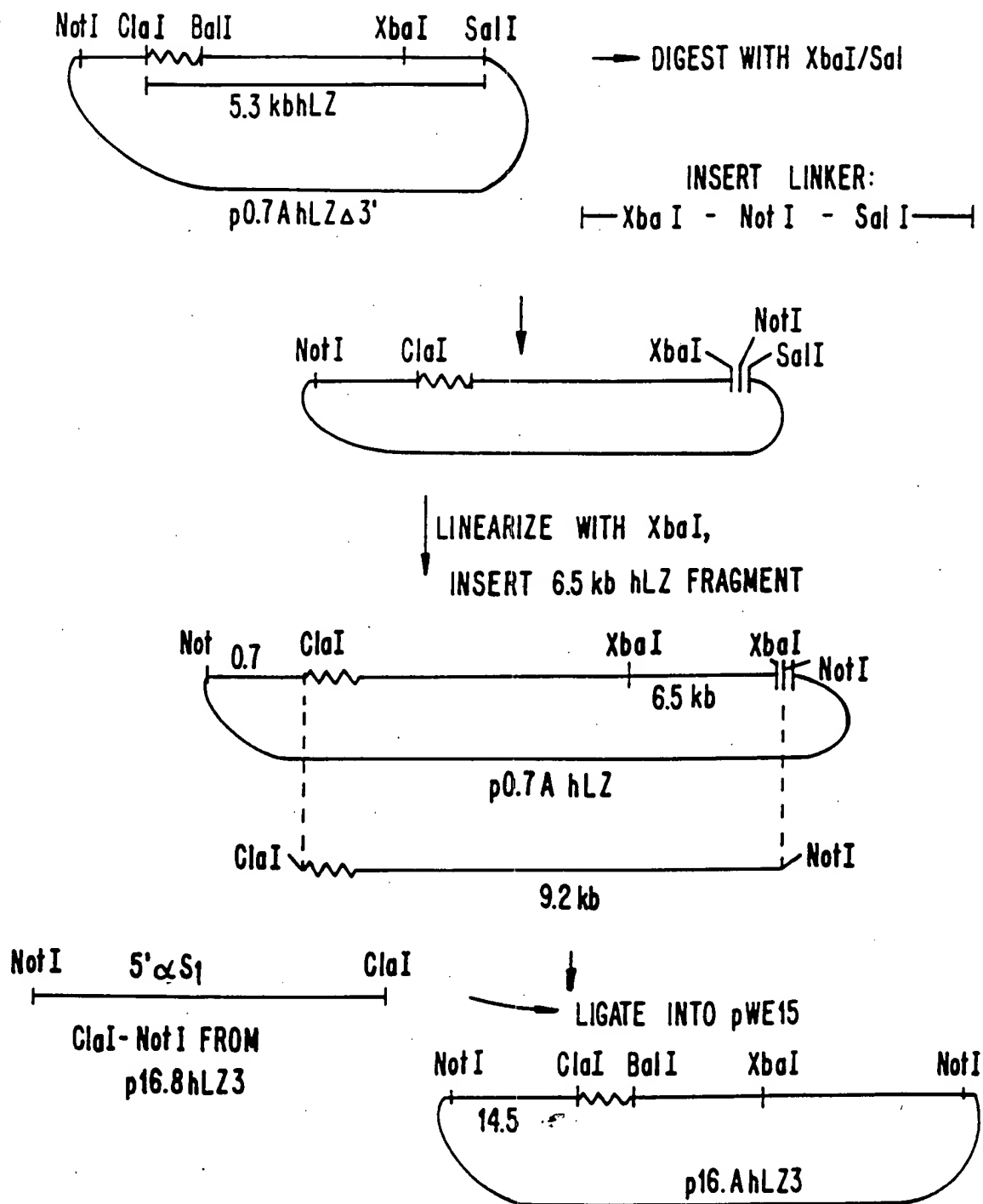


FIG. 27.